REMARKS

Upon entry of the foregoing amendments, claims 21-23, 26, and 28-38 are under consideration. Claims 15-19 were canceled without prejudice or disclaimer as directed to non-elected inventions. Support for the amendment to claims 28, 33 and 33 is found in the specification at page 8, lines 3-6. No new matter is added.

Restriction Requirement

In response to the Restriction Requirement dated November 20, 2001, Applicants elect the invention of Group I (claims 21-26 and 28), drawn to a dimerized fusion protein, without traverse.

§ 112, First Paragraph Rejection: Written Description

1. The Examiner has rejected claims 25-26, and 28 under 35 USC § 112 first paragraph for lack of written description. The Examiner asserts that the specification and the claims as originally filed does not provide support for the invention as now claimed. The Examiner states that claim 25 is still pending. In response, Applicants note that claim 25 has been canceled herein. Therefore, this rejection is moot as it pertains to claim 25. The remaining rejections are addressed as follows.

1A. "more Galα1, 3Gal epitopes than a wild-type P-selectin glycoprotein ligand-1"

The Examiner states that "Applicant argues that the experiment disclosed on page 12 of the specification supports the claim to the fusion protein of claim 21, wherein the first polypeptide comprises more Galα1, 3Gal epitopes than a wild-type P-selectin glycoprotein ligand-1." (See Final Office Action, page 2). In response, Applicants assert that pending claim 21 does not recite the phrase "more Galα1, 3Gal epitopes than a wild-type P-selectin glycoprotein ligand-1." Applicants would like to note to the Examiner that claim 26, which depends from claim 21, does not recite this phrase. Applicants further note that claim 28, which depends from claim 21, as ameded herein recites in part, "wherein the first polypeptide comprises more Galα1, 3Gal epitopes than the human wild-type P-selectin glycoprotein ligand-1 polypeptide." Applicants address this rejection as it applies to claim 28. The specification

discloses at, *e.g.*, page 11, lines 12-37 and Figure 1, that fusion proteins containing the human PSGL-1 polypeptide that are glycosylated by an α1,3 galactosyltransferase contain more Galα1, 3Gal epitopes than the human wild-type P-selectin glycoprotein ligand-1 polypeptide, as shown by Western blotting with the *Bandereria simplicifolia* isolectin B_4 (See Figure 1, right column). Applicants have amended claim 28 herein to recite the phrase "wherein the first polypeptide comprises more Galα1, 3Gal epitopes than the human wild-type P-selectin glycoprotein ligand-1 polypeptide." Applicants assert that the recitation of the phrase "the human wild-type P-selectin glycoprotein ligand-1 polypeptide" is a single species, and thus, that one skilled in the art could readily determine if the claimed polypeptide falls within its scope. Thus, claim 28 as amended herein is fully supported by the as filed specification. Therefore, this rejection should be withdrawn.

2. The Examiner has rejected claims 21-23, 25-26, and 28-38 under 35 USC § 112 first paragraph for lack of written description. The Examiner asserts that the specification and the claims as originally filed does not provide support for the invention as now claimed. Claim 25 has been canceled herein. Therefore, this rejection is moot as it pertains to claim 25. The remaining rejections are addressed as follows.

2A. "an immunoglobulin heavy chain polypeptide"

Regarding claims 21, 25, 26, and 29, the Examiner states that the specification and the claims as originally filed do not provide support for the phrase "an immunoglobulin heavy chain polypeptide." Applicants have canceled claim 25. Thus, this rejection is moot as it applies to this claim. Applicants traverse this assertion to the extent it applies to claims 21, 26 and 29. Applicants assert that the specification at page 8, lines 6-10, recites that "[t]he mucin/immunoglobulin expression plasmid was constructed by fusing the PCT-amplified cDNA of the extracelluar part of PSGL-1 in frame via a BamHI site, to the Fc part (hinge, CH2 and CH3) of mouse IgG_{2b} carried as an expression casette in CDM7." It is known to one of ordinary skill in the art that the Fc (fragment crystallizable) region of an IgG inherently contains an immunoglobulin heavy chain polypeptide, particularly since the immunoglobulin light chain polypeptide is not present in the Fc region, as it is contained within the F(ab) region of an IgG. (See, *e.g.*, Figure 1, Chapter 9, pages 209-233 of Fundamental Immunology, 2nd Edition, W.E. Paul, *ed.*, Raven Press, NY; courtesy copy enclosed).

A structure or process not explicitly described may meet the conveyance standard if it is "inherent" in what is described. See Standard Oil Co. v. Montedison, S.p.A., 494 F.Supp 370 (D. DE 1980) ("[p]atent entitlement is based on scientific skill and diligence and not on the ability to manipulate the English language, . . . Legal equivalence, or inherency, may be established either by the direct meaning of the language or by inferenced drawn from the terms of the initial disclosure." In the pending application, one skilled in the art would reasonably conclude that Applicant's disclosure of the Fc region of an IgG inherently discloses an immunoglobulin heavy chain polypeptide, and, therefore, that the Applicants had possession of the claimed invention at the time the application was filed. Thus, pending claims 21, 26, and 29 are fully supported by the as filed specification.

2B. "the extracellular portion of a P-selectin glyoprotein ligand-1"

Regarding claim 29, The Examiner states that the specification and the claims as originally filed do not provide support for the phrase "extracellular portion of a P-selectin glyoprotein ligand-1." Applicants traverse. The specification at page 8, lines 6-8, recites that "[t]he mucin/immunoglobulin expression plasmid was constructed by fusing the PCR-amplified cDNA of the extracelluar part of PSGL-1 in frame via a BamHI site." (Emphasis added). The plain meaning of the term "part" is "a division, portion or segment of a whole." (Webster's II New Riverside Dictionary, Revised, 1996). "Patent entitlement is based on scientific skill and diligence and not on the ability to manipulate English synonyms." Standard Oil Co. v. Montedison, S.p.A. 494 F. Supp. 370, 384 (D. Del. 1980). Therefore, the phrase "extracellular portion of a P-selectin glyoprotein ligand-1" as used in claim 29 is fully supported by the as filed specification.

2C. "more Galα1, 3Gal epitopes than a wild-type P-selectin glycoprotein ligand-1"

Regarding claims 33 and 38, The Examiner states that the specification and the claims as originally filed do not provide support for the phrase "comprises more Galα1, 3Gal epitopes than a wild-type P-selectin glycoprotein ligand-1." In response, Applicants note that claims 33 and 38 have been amended herein to recite "comprises more Galα1, 3Gal epitopes than the human wild-type P-selectin glycoprotein ligand-1 polypeptide." (Emphasis added). As noted above, the specification discloses at, *e.g.*, page 11, lines 12-37 and Figure 1, that fusion proteins containing the human PSGL-1 polypeptide that are glycosylated by an α1,3 galactosyltransferase

contain more Galα1, 3Gal epitopes than the human wild-type P-selectin glycoprotein ligand-1 polypeptide, as shown by Western blotting with the *Bandereria simplicifolia* isolectin B_4. Therefore, claims 33 and 38 as amended herein are fully supported by the as filed specification.

2D. "comprises a part of a P-selectin glycoprotein ligand-1 that mediates binding to selectin"

Regarding claim 34, The Examiner states that the specification and the claims as originally filed do not provide support for the phrase "comprises a part of a P-selectin glycoprotein ligand-1 that mediates binding to selectin." In response, Applicants assert that claim 34 is fully supported; the specification at page 4, lines 34-36, recites "in a preferred embodiment, the antigenic fusion protein according to the invention further comprises a part, which mediates binding to selectin, such as P-selectin." The specification further discloses at page 5, lines 6-9 that "the part that mediates binding to selectin is the P-selectin glycoprotein ligand-1 (PSGL-1) or an essential part thereof." Therefore, pending claim 34 is fully supported by the as filed specification. These rejections can be withdrawn.

CONCLUSION

Applicants believe that the claims, as amended, are in condition for allowance. If the Examiner has any questions, the Examiner is invited to contact the undersigned by telephone.

Respectfully submitted,

42,764

Dated: April 11, 2003

Ivor R. Elrifi, Reg. No. 39,529
Cynthia Kozakiewicz, Reg. No. 42,764
Attorneys for Applicant
c/o MINTZ, LEVIN
One Financial Center
Boston, MA 02111
Tel: (617) 542 6000
Fax: (617) 542 2241

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Claims 28, 33 and 38 have been amended as follows:

28. (Amended) The fusion protein of claim 21, wherein the first polypeptide comprises more Galα1, 3Gal epitopes than the human [a] wild-type P-selectin glycoprotein ligand-1 polypeptide.

33. (Amended) The fusion protein of claim 29, wherein the first polypeptide comprises more Galα1, 3Gal epitopes than the human [a] wild-type P-selectin glycoprotein ligand-1 polypeptide.

38. (Amended) The fusion protein of claim 34, wherein the first polypeptide comprises more Galα1, 3Gal epitopes than the human [a] wild-type P-selectin glycoprotein ligand-1 polypeptide.

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Fundamental Immunology, Second Edition, edited by William E. Paul, Raven Press Ltd., New York © 1989.

9

Immunoglobulins: Structure and Function

Charles A. Hasemann and J. Donald Capra

Department of Microbiology and Program in Immunology, The University of Texas Southwestern Medical Center, Dallas, Texas 75235

The relationship between the structure and function of the immunoglobulin molecule is a tribute to the power of molecular evolution. Via the duplication and diversification of the immunoglobulin homology domain, a family of molecules with diverse biological functions has been derived (1–3). The seemingly paradoxical relationship between uniformity and diversity is one of the things that makes the study of immunoglobulins interesting and rewarding.

Immunoglobulins are the prototype members of the immunoglobulin supergene family (4). This family of molecules shares a conserved protein sequence that represents the duplication of a primordial gene segment. As is made clear later, it is the linear combination of two or more of these segments that forms an immunoglobulin heavy or light chain. There are several other molecules that are also derived from this primordial sequence element, such as the CD4 and CD8 molecules (see Chapter 4), the antigen specific chains of the T cell receptor (see Chapter 11), and both the class I and class II MHC antigens (see Chapter 17) to name a few. Three-dimensional analyses of molecules that contain these immunoglobulin-like domains have revealed that there is more than simply an amino acid sequence homology between them; this conserved sequence represents a structural motif that can be used as a subunit in building large macromolecules (5,6). Thus there has been conservation of this immunoglobulin homology domain throughout evolution such that not only are immunoglobulin heavy and light chains strikingly similar to each other, but similar to a variety of other molecules as well.

As similar in structure as these molecules are, variation is the underlying essence of antibody function. Through the switch from one heavy chain isotype to another, the functional nature of the immune response can be altered significantly (see Chapter 14). Complement fixation, the ability to cross the placenta, and the ability to form multimers are just a few of the variations in antibody function due to isotypic differences (7–9). Each of these different biological responses is due to discreet differences in antibody structure.

The truly remarkable feature of antibody function, however, is the ability to recognize specific antigenic determinants. The potential repertoire of distinct antibody

specificities is, for all practical purposes, infinite. This function is attributed to the variable domains of the heavy and light polypeptide chains of any given antibody (10). While called the "variable" region, any two variable regions are likely to be from 70 to 99% identical, and even those that have only a single amino acid difference can demonstrate distinct antigenic specificities. Thus, through only slight variations in structure, the entire antibody repertoire of an individual is determined. The intent of this chapter is to emphasize the details of antibody structure which allow this simple class of molecules to function in such a variety of elegant ways.

IMMUNOGLOBULIN STRUCTURE

General Immunoglobulin Features

A prototypic immunoglobulin molecule is composed of four polypeptide chains which are joined into a macromolecular complex via several disulfide bonds (5). Figure 1 is a diagram of such a prototypic molecule. The smaller polypeptide is called a light chain and the larger a heavy chain. An antibody molecule is composed of two identical light chains and two identical heavy chains. The exclusive use of one heavy chain sequence and one light chain sequence is the consequence of allelic exclusion, a genetic event that is detailed in Chapter 10. The net result of allelic exclusion is that only one light chain gene and one heavy chain gene are expressed in any antibody-producing cell, and thus two identical heavy chain polypeptides and two identical light chain polypeptides are assembled into a single immunoglobulin molecule.

Based on experiments with proteolytic enzymes such as papain or trypsin, which cleave the immunoglobulin molecule at specific points, the molecule can be divided into two basic functional domains (10). As Fig. 1 shows, there are two F_{ab} fragments consisting of a light chain and a fragment of the heavy chain. The name F_{ab} is derived from the fact that this portion of the molecule contains the antigen-binding (ab) activity of the molecule. The remaining portion, the F_c fragment, is so named because it is easily crystallized (c). The F_c portion of the molecule is responsible for the biological effector functions of the immunoglobulin molecule, such as complement fixation. Another proteolytic fragment, F(ab)$'_2$ consists of the two F_{ab} fragments plus the portion of the heavy chain that contains one or more interchain disulfide bonds. Thus the F(ab)$'_2$ fragment is divalent, unlike the F_{ab} fragment which is univalent.

Early studies of immunoglobulin structure often used the immunoglobulin as an antigen (reviews in refs. 11 and 12). Human myeloma proteins, the antibody product of a plasma cell malignancy (and later, mouse plasmacytomas and hybridomas), were used as a homogeneous source of immunoglobulin for these studies. In this way heterologous antisera were created which, after several adsorption steps, would subdivide immunoglobulins into a finite number of groups. These groups, termed isotypes, are the serological consequence of the fact that there are

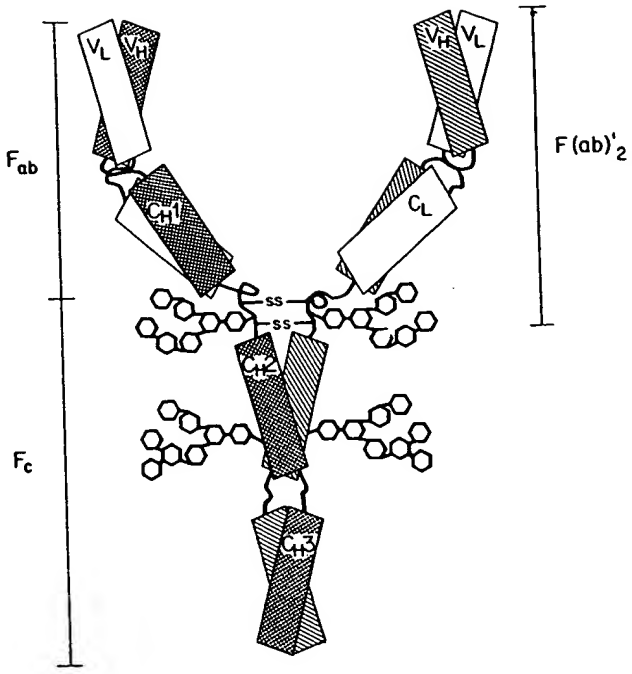


FIG. 1. Diagram of a prototypic immunoglobulin monomer. Each *rectangle* represents an immunoglobulin domain, with the extended polypeptide strands connecting the domains into complete heavy (*dark shading*) and light (*light shading*) chains. The interchain disulfide bonds between the hinge regions of the heavy chains are represented as *black bars*. Intrachain and interchain disulfides between heavy and light chains are not shown. Carbohydrate groups are shown connected to both the hinge regions and the second constant region domain of each heavy chain. The boundaries of the major proteolytic fragments are indicated by the *bars* to the **left** and **right** of the figure. Note that the F_{ab}/F_c division occurs above the interchain disulfides, while the F(ab)$'_2$ division is below.

several different kinds of heavy and light chain constant regions. There are five heavy chain classes, designated by the Greek letters mu (μ), delta (δ), gamma (γ), alpha (α), and epsilon (ε). There are two light chain types, kappa (κ) and lambda (λ). The isotype of a given antibody molecule depends on the class of heavy chain and the type of light chain that are used. A common notation to designate the isotype of an antibody is to follow the antibody name with the appropriate Greek letters, for example, MOPC104E (μλ). Since the effector functions of the antibody are a consequence of the heavy chain only, it is often sufficient to refer to the heavy chain class alone. Thus IgM refers to antibodies utilizing a μ chain, IgG for γ, and so on. The use of immunoglobulins as antigens, and the various antigenic markers that immunoglobulins bear, are discussed further in the final section of this chapter.

Each heavy and light chain can be divided into domains, each domain consisting of approximately 110 amino acids. The light chain consists of two domains, indicated as V_L and C_L (Fig. 1). V_L is the variable region of the light chain and is the region of the light chain which participates in

antigen binding. C_L is the constant region of the light chain, which is essentially invariant for a given light chain type. The heavy chain also has a variable region, V_H, which as in the light chain is the portion of the polypeptide that participates in antigen binding. In contrast to the light chain, the prototype heavy chain has three constant domains, C_H1, C_H2, and C_H3. In addition, the heavy chains of most immunoglobulins have a region known as the hinge (generally located between C_H1 and C_H2), which give the F_{ab} portions of the molecule considerable freedom to move about in relation to the F_c. In both heavy and light chains the variable and constant regions are encoded by separate genes (13). This means that any V_H or V_L region can be combined with any C_H or C_L region, respectively. Thus the ability to recognize a given antigen (a V region property) can be linked to any of the various effector functions (a C region property).

As indicated previously, each of these V and C domains bears a sequence homology that indicates that they were at one time derived from a common ancestral gene. One important feature of this domain structure is the presence of two cysteines which form an intramolecular disulfide bond. With rare exceptions, there is one disulfide bond per domain, and this is always an intradomain bond. This bond is thought to be important for maintaining the tertiary structure of the immunoglobulin subunit as described in a later section. Disulfide bonds are important to the quaternary structure of the immunoglobulin molecule as well (14). The light chain is generally attached to a heavy chain by a disulfide bond, and, in turn, the heavy chains are covalently linked to one another by disulfide bridges between the hinge units. This pattern of disulfide bonding results in a molecule that can be thought of as composed of two identical half molecules, each consisting of a single heavy and light chain pair. Each heavy and light chain pair has the capacity to recognize and bind the same epitope. Thus an intact immunoglobulin molecule can interact with two epitopes simultaneously. This characteristic of immunoglobulin structure gives an immunoglobulin monomer a binding valence of two. Experimental determinations of the actual valence of immunoglobulin monomers give a value slightly less than two. This difference is believed to be due to steric hindrance and other thermodynamic considerations.

Immunoglobulins also occur in multimers, which are covalently bound concatamers of the basic immunoglobulin monomer. Not all isotypes of immunoglobulin form multimers. Specifically, IgA usually forms a dimeric molecule, and IgM forms a pentameric molecule. Both of these macromolecules utilize an accessory molecule called J chain to form these complexes (reviewed in ref. 15). Immunoglobulin multimers have higher valences for antigen binding roughly proportional to the additional number of binding sites in the molecule. In addition, the secreted forms of IgA are associated with a molecule called secretory component (SC), which is involved in immunoglobulin transport across epithelial membranes (16).

Finally, immunoglobulins are glycoproteins and, with some exceptions, glycosylation is restricted to the constant region of the heavy chain. Different heavy chain classes have different types of carbohydrate groups and different locations of carbohydrate attachment. The prototypic molecule shown in Fig. 1 has carbohydrate attached both in the hinge region and in the second constant region domain. This carbohydrate is thought to be important for correct immunoglobulin folding and transport during synthesis (17) and appears to regulate the turnover rate of immunoglobulin. No effector functions have been directly attributed to carbohydrate. In the section on heavy chains to follow, variation in carbohydrate content is discussed more completely.

The Immunoglobulin Domain

As mentioned previously, immunoglobulins are composed of the linear combination of a basic subunit structure. This subunit, or domain, has a compact globular structure in three dimensions. Figure 2 is a schematic representation of data derived from X-ray diffraction studies of an immunoglobulin light chain (18). The solid arrows represent the seven polypeptide strands that comprise the antiparallel β-pleated sheets in an immunoglobulin domain. Each domain contains two such β-pleated sheets, one β sheet consisting of four β strands, the other consisting of three β strands. These β sheets form a "hydrophobic sandwich" between them. The numbering of the β strands reflects which layer the strand is in, either the four stranded sheet or the three. The two β sheets are covalently linked by a disulfide bond. The half cystines that form this bond are conserved in all molecules that possess immunoglobulin domains. The loops that connect the β strands are frequently glycine rich, which increases their flexibility. This structural motif, two β sheets forming a barrellike structure with a hydrophobic core, is referred to as the "immunoglobulin fold" or more generally a "β barrel." All the immunoglobulin constant region domains maintain this same basic structure. The variable region domains have a slightly different structure, wherein several of the loops connecting the β strands (those at the right end of Fig. 2) are somewhat longer.

The polypeptide strand connecting the V and C (C_L or C_H1) domains is called the switch. The switch is important to antibody structure because of the flexibility it allows between the V and C domains. One particularly important feature of this flexibility is that it allows the two domains to rotate relative to one another. Figure 2 demonstrates the importance of this rotation; note that the four-stranded β sheet of the C domain is on top, while in the V domain, the three-stranded sheet is on top. When a heavy chain and light chain combine to form an intact H–L pair as depicted in Colorplate 1 (see page 220), the C_H and C_L domains make close contacts between the two four-stranded sheets, while V_H and V_L pair via the three-stranded sheets (19). The C_H–C_L pairing creates a compact hydrophobic core between the β sheets forming an anchor for the V domains. The V_H–V_L pairing has a more hydrophilic groove formed by the three-stranded sheets forming a pocket in which small molecules can fit. This groove, together with the loops at the end of the V regions, form the antibody-combining site for antigen.

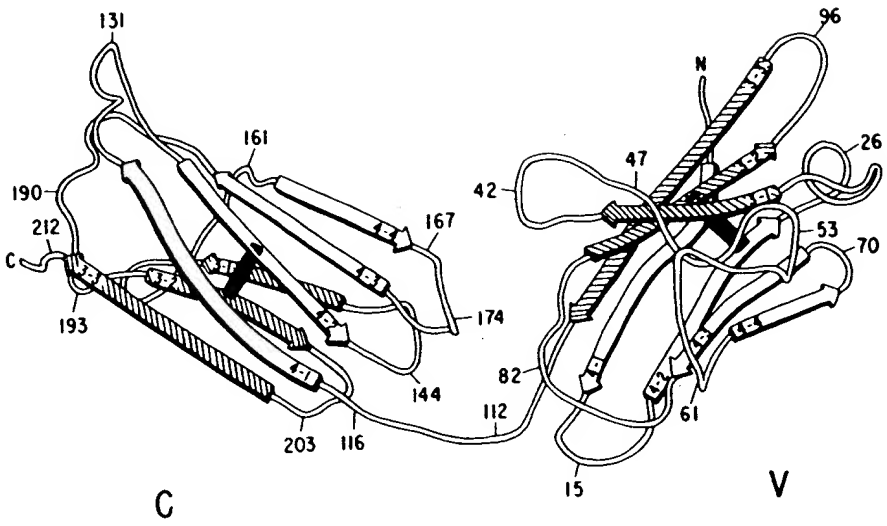


FIG. 2. Schematic drawing of the V and C domains of a light chain. The β strands participating in the antiparallel β-pleated sheets of each domain are represented as *arrows*. The β strands of the three-stranded sheets are *shaded*, while those in the four-stranded sheets are *white*. The β strands are numbered according to the scheme of Edmundson. The intradomain disulfide bonds are represented as *black bars*. Selectec amino acids are numbered, with position 1 as the N terminus. (From Edmundson et al., ref. 18, with permission.)

It is clear from this background of immunoglobulin structure that the three-dimensional structure of the immunoglobulin fold places a considerable constraint on the primary structure of immunoglobulin domains. The following sections examine the primary structures of heavy and light chains, and how several immunoglobulin domains combine to form distinct quaternary structures with distinct biological functions.

Heavy Chains

Heavy chains can be divided into three functional regions: F_d, hinge, and F_c. F_d in combination with a light chain forms F_{ab}, which has all the antigen-binding properties of an intact immunoglobulin. F_d can be subdivided further into the V_H and C_H1 domains, wherein V_H possesses all the antigen-binding properties and C_H1 acts mostly as an anchor (holding the variable region stable) and as a spacer (moving the antigen-binding portion of the molecule further from the F_c fragment). The hinge acts as a more flexible spacer, allowing the F_{ab} fragments to move more freely in space. Finally, the F_c fragment bears the effector functions of the immunoglobulin molecule. Each F_c fragment can occur in two forms, the membrane and secreted forms. The difference between these forms is found in the carboxy terminus, with the membrane form having a long hydrophobic stretch which anchors the polypeptide in the cell membrane. These alternate forms are achieved via alternative mRNA processing from the same primary transcript (see Chapter 10).

Constant Regions

The constant regions of the human heavy chains, including two subclasses of α (α_1, α_2), are diagrammed in Fig. 3. Subclasses represent a more recent duplication of a constant region gene. The α class has two subclasses in the human (α_1, α_2), but the distribution of subclasses varies in other species. The most apparent variations in heavy chain structure involve the hinge region and carbohydrate attachments, although more subtle structural differences can be found in the primary structures of C_H and F_c.

C_H1 and F_c Regions

The protein sequences of the C_H1 and F_c regions of the five human immunoglobulin classes (including γ subclasses γ_1 and γ_3) are presented in Fig. 4. The sequences have been aligned to display the homology of the sequences to one another. Positions that are invariant or highly conserved between the six sequences are indicated. The positions of the β strands are placed and numbered according to the scheme of Edmundson et al. (18). There are three amino acids that are invariant in all three domains: the two cysteines that form the intrachain disulfide bond and a tryptophan that is thought to protect the disulfide bond from reduction by solvent. It is clear that the most conserved amino acids between the classes lie in the β strands. This observation is repeated in the data presented in Fig. 5, where the four domains of the μ chain are aligned with the κ and λ constant regions. The overall homology between these domains is approximately 22%, with nearly all the homologous residues centered around the invariant cysteines and tryptophan and in the β strands. Note in Fig. 4 that the F_c sequences of μ and α have 18 extra amino acids at their carboxyl end. This extra segment is the site of J chain attachment involved in the formation of IgA and IgM multimers (21).

The overall identity of the F_c regions of the various classes is approximately 30%. The identity of γ and ε is significantly higher than this average. Different domains show different interclass homologies, the C_H1 domain being most similar at 33%, probably a reflection of the common function of pairing with immunoglobulin light chains. At an average identity of 41%, the carboxy terminal domains of μ, α, and γ are significantly more relate

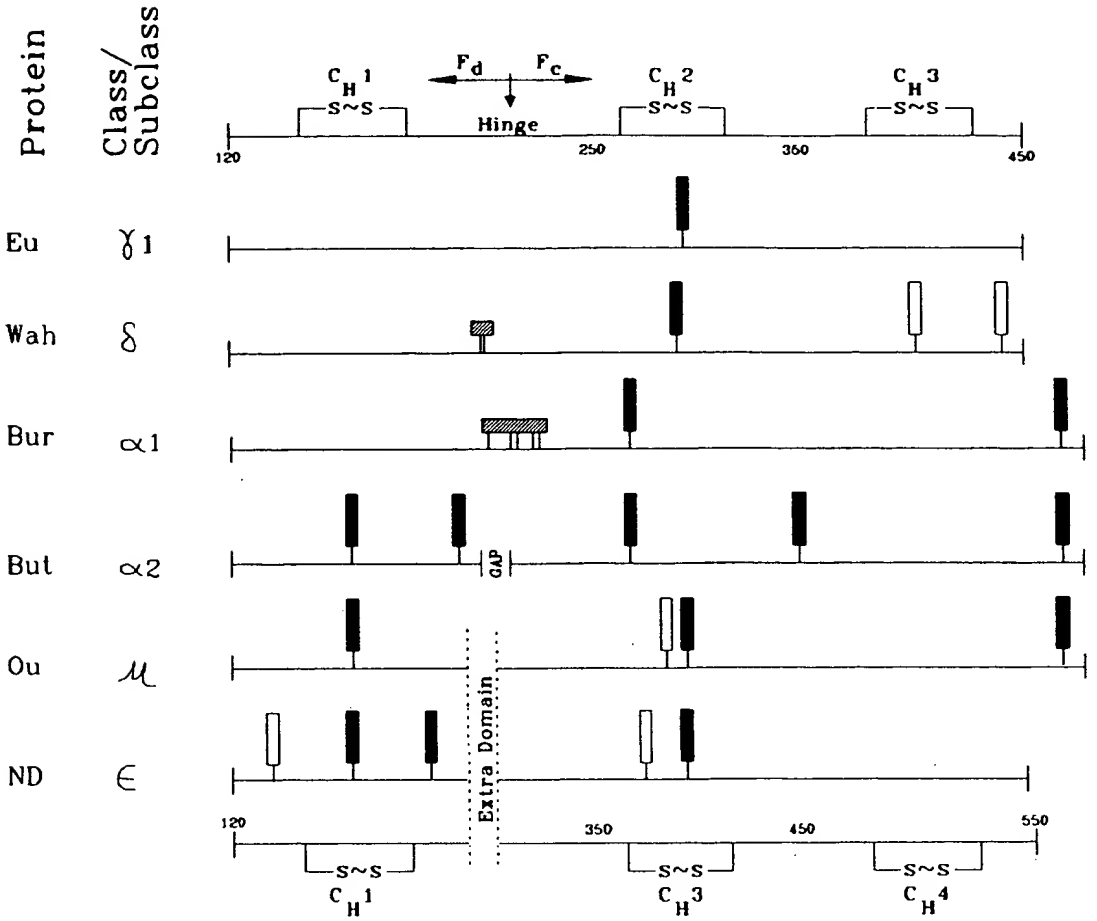


FIG. 3. Comparison of the major features of the five human heavy chain classes including the α_1 and α_2 subclasses. The **upper scale** gives amino acid positions and domain features for the top four chains; the **lower scale** applies to the μ and ϵ chains, which consist of four C_H domains. Note that the C_H2 domain is omitted from the latter two chains. Positions of the oligosaccharide substituents of the various chains are indicated by *rectangles, shaded* if found in homologous positions in two or more molecules. Glucosamine oligosaccharides are represented by *vertical rectangles*, galactosamine oligosaccharides by *horizontal rectangles*. (From Torano et al., ref. 20, with permission.)

than the average for the entire F_c. Between the μ and α domains this conservation of sequence identity is probably related to their common role in multimer formation. These preceding observations suggest that the domains of the five immunoglobulin classes have evolved at different rates and along different paths (22). These structural differences undoubtedly play a role in the various biological functions of the five immunoglobulin classes. This idea is further supported by the fact that the interspecies homology between classes is higher than the interclass homologies in the same species (compare Figs. 4A and 6).

Finally, it is important to remember that the F_c region has a three-dimensional structure and that the conservation and divergence of the F_c sequence is reflected in the position of amino acids in this structure. Colorplate 2 (see page 220) shows the α-carbon backbone of human IgG F_c (23). In each domain certain amino acids are exterior and important in a functional sense due to their accessibility to other molecules. Other amino acids are interior, acting as structural elements. Differences in the primary sequences of the immunoglobulin domains can affect either of these functions, ultimately changing the functional phenotype of the heavy chain class.

Hinge Regions

The hinge regions display the greatest amount of interclass variability. Figure 7 represents an alignment of the hinge regions from several human immunoglobulins. Human μ and ϵ chains do not have a hinge region, but rather an entire immunoglobulin domain designated $C_\mu2$ and $C_\epsilon2$, respectively. The positions of the seven β strands are indicated as in Fig. 4. It is thought that hinge regions evolved from the μ/ϵ C_H2 domain, although the homologies are too low to be certain. The hinges from δ and γ_1 are aligned with the μ and ϵ domains to illustrate this point in Fig. 7A.

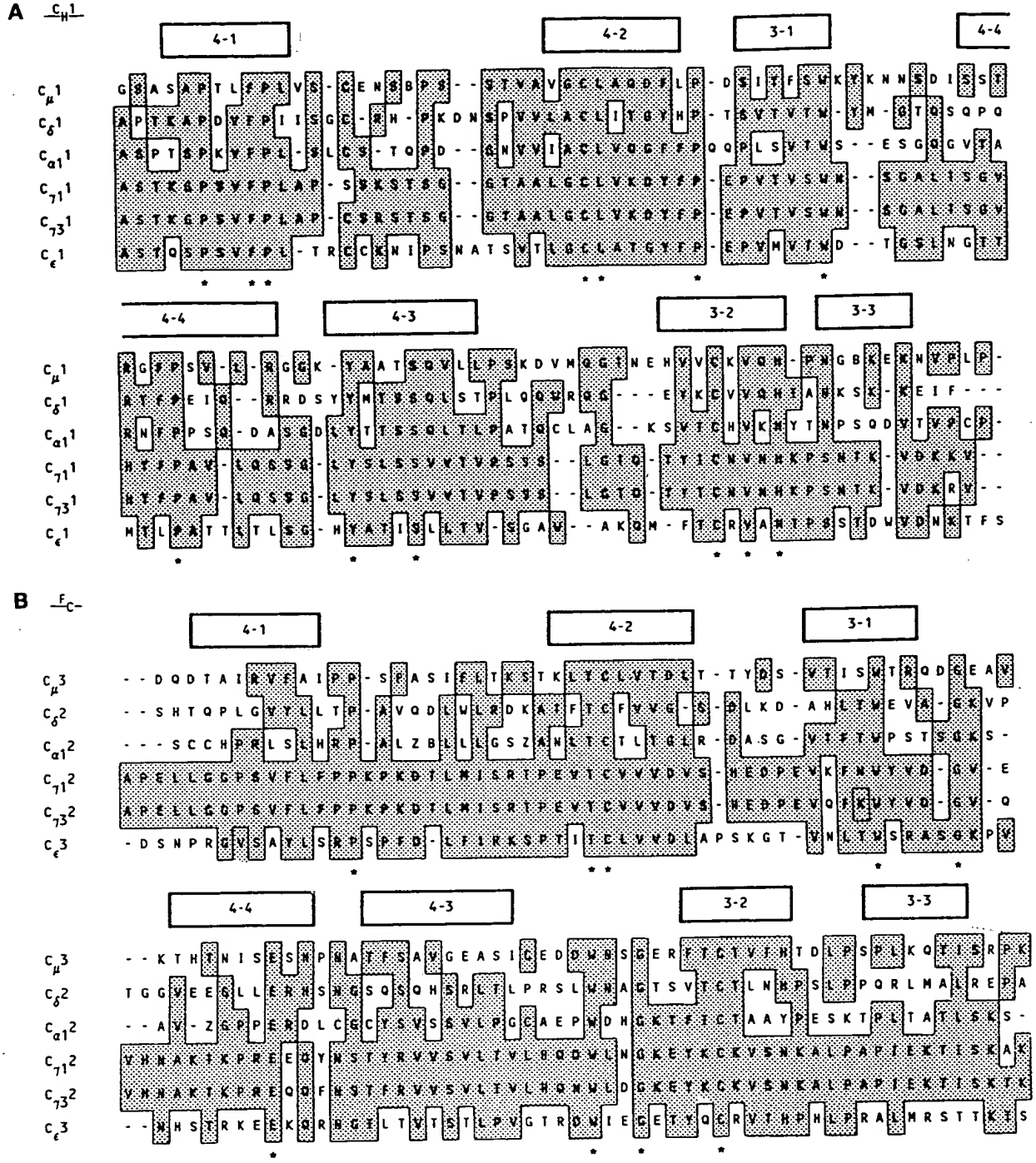


FIG. 4. Comparison of the amino acid sequences of the C_H1 domains **(A)** and F_c regions **(B)** of the five classes and γ_1 and γ_3 subclasses of human immunoglobulins. The sequences are aligned as a compilation of the pairwise comparison of each chain to one another using the AALIGN program [part of the DNAstar molecular biology analysis system (DNASTAR, Inc., 1801 University Ave., Madison, WI 53705)], introducing gaps (—) to achieve maximal homology. Amino acid sequences that are identical between two or more sequences have been *boxed* and *shaded* to emphasize the overall homology. Invariant amino acids within each domain are indicated by an *asterisk* beneath the sequences. The positions of the β strands are indicated above each domain, with the numbering of the β strands per Edmundson as in Fig. 2.

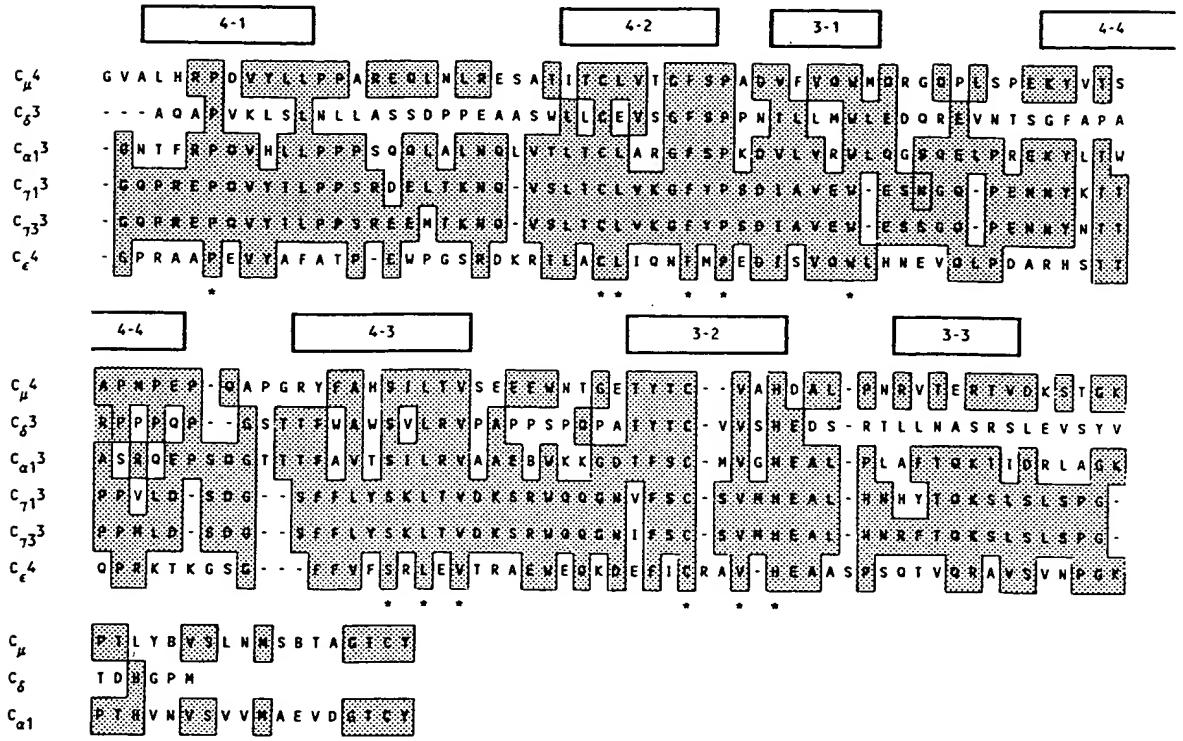


FIG. 4B. *Continued.*

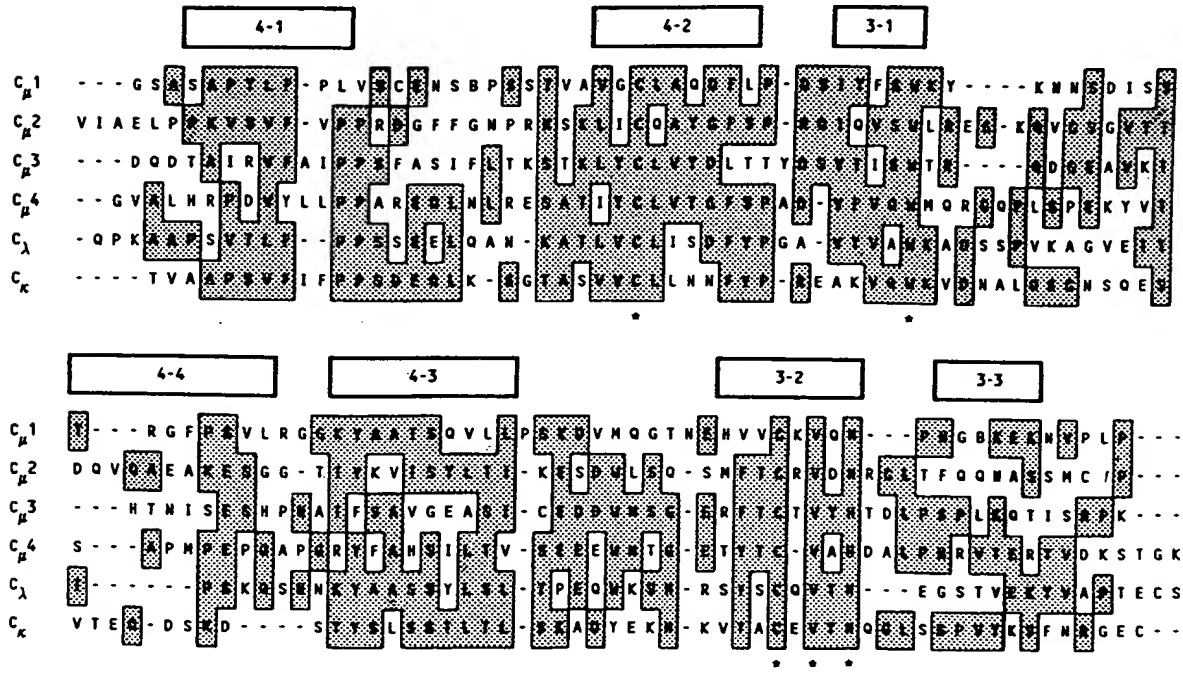


FIG. 5. Comparison of the amino acid sequences of the four μ domains and the C_λ and C_κ domains of human immunoglobulins. Sequences aligned as described in Fig. 4. Positions of the β strands as described in Fig. 2. Extensive gapping was required to achieve this level of homology.

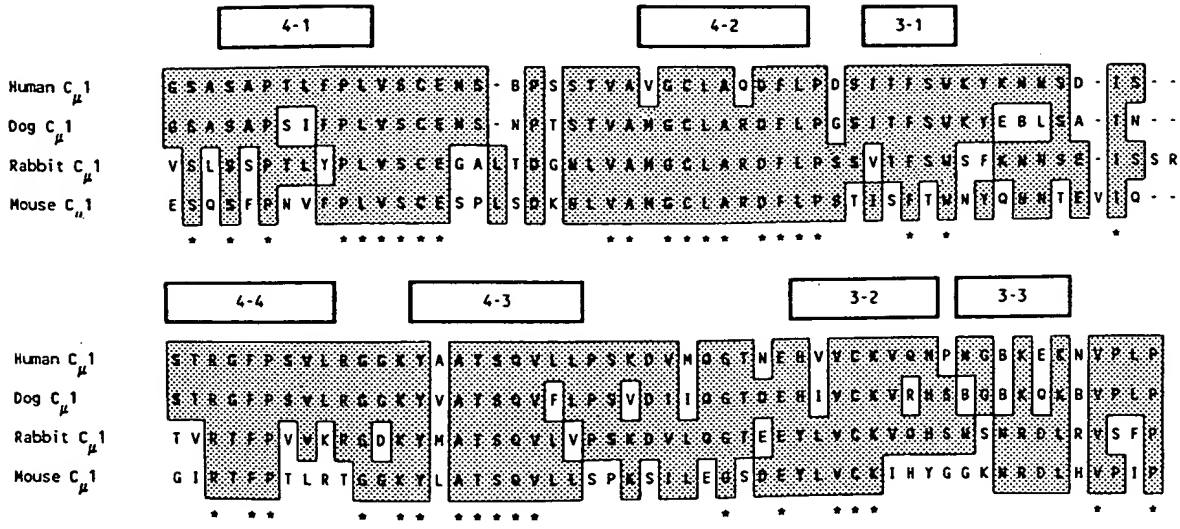


FIG. 6. Comparison of the amino acid sequences of the $C_\mu 1$ domains from four species. Sequences aligned as described in Fig. 4. Positions of the β strands as described in Fig. 2. Little gapping was required in this comparison. Note that 40 amino acid positions designated by * are invariant between the $C_\mu 1$ domains of these four distant species.

The protein sequences of the hinge regions also display very little sequence homology between classes, with most if not all identities owing to the high frequencies of proline and cysteine residues in this region (Fig. 7B). The relationship of the hinge regions within the subclasses is particularly interesting, however. The γ_1 hinge contains the sequence, C–P–P–C, in which the cysteines form interchain (H–H) disulfide bonds. The resulting cyclic octapeptide

$$\cdots C\text{–}P\text{–}P\text{–}C\cdots$$
$$\quad\;|\qquad\;|$$
$$\cdots C\text{–}P\text{–}P\text{–}C\cdots$$

is thought to act as a pivot, giving the hinge a very flexible structure. Comparison of the γ_3 and γ_1 hinge region sequences reveals that γ_3 contains four duplications of the γ_1 hinge sequence. The presence of four cyclic octapeptides in the γ_3 hinge is thought to make the γ_3 hinge exceptionally flexible, both due to its length and the presence of several pivots (24,25).

Both the δ and α_1 hinge regions contain sites for the attachment of carbohydrate moieties. The δ hinge can be subdivided into two regions (26). The amino proximal region contains five carbohydrate attachment sites and bears significant sequence homology to the $C_\mu 2$ domain. This region of the hinge is resistant to proteolytic digestion. The carboxy terminal portion of the hinge region is highly charged and predicted to form an α-helical structure. This region of the hinge is quite susceptible to proteolytic digestion, which is probably responsible for the short half-life of IgD in the serum. The murine δ hinge lacks the α-helical portion of the human δ hinge which might indicate a different biological role for the two IgDs.

The α_1 hinge also contains five carbohydrate attachment sites, but in a stretch of only 17 amino acids (27). This makes the α_1 hinge exceptionally resistant to cleavage by intestinal proteases, an advantageous property for IgA, considering its role as the secretory immunoglobulin. Several strains of bacteria produce extracellular proteolytic enzymes which specifically cleave IgA_1 in the hinge sequence. The α_2 hinge has a deletion of 13 amino acids as compared to the α_1 hinge, making it resistant to the bacterial enzymes (28). Thus it would appear that the differences in the α hinges represent a mechanism to avoid proteolytic degradation of two different types.

Carbohydrate

The number and position of carbohydrate moieties in immunoglobulin heavy chains is quite variable. There are, however, several carbohydrate groups that are found in analogous positions in several different classes. Carbohydrate is found almost exclusively in heavy chain constant regions, although it is present in approximately 15% of variable regions, whenever the carbohydrate acceptor sequence Asn-X-Ser/Thr is present (29). The positions of the carbohydrate substituents found in the human heavy chains are indicated in Fig. 3. Note that both complex (N-linked) and simple (O-linked) carbohydrate groups are found in immunoglobulin. Carbohydrate groups, which are found at homologous positions in more than one class, are indicated with a black box.

The exact role of carbohydrate in immunoglobulin function is unknown. The fact that homologous heavy chain classes between species have preserved a great deal of the carbohydrate substituents argues in favor of an important structural or functional role for carbohydrate. Possible functional roles include (a) tertiary structure—as can be seen in Colorplate 2, the carbohydrate in the IgG heavy chain acts as a spacer in the center of the F_c fragment; (b) immunoglobulin synthesis and secretion—when carbohydrate attachment is inhibited, both IgA and

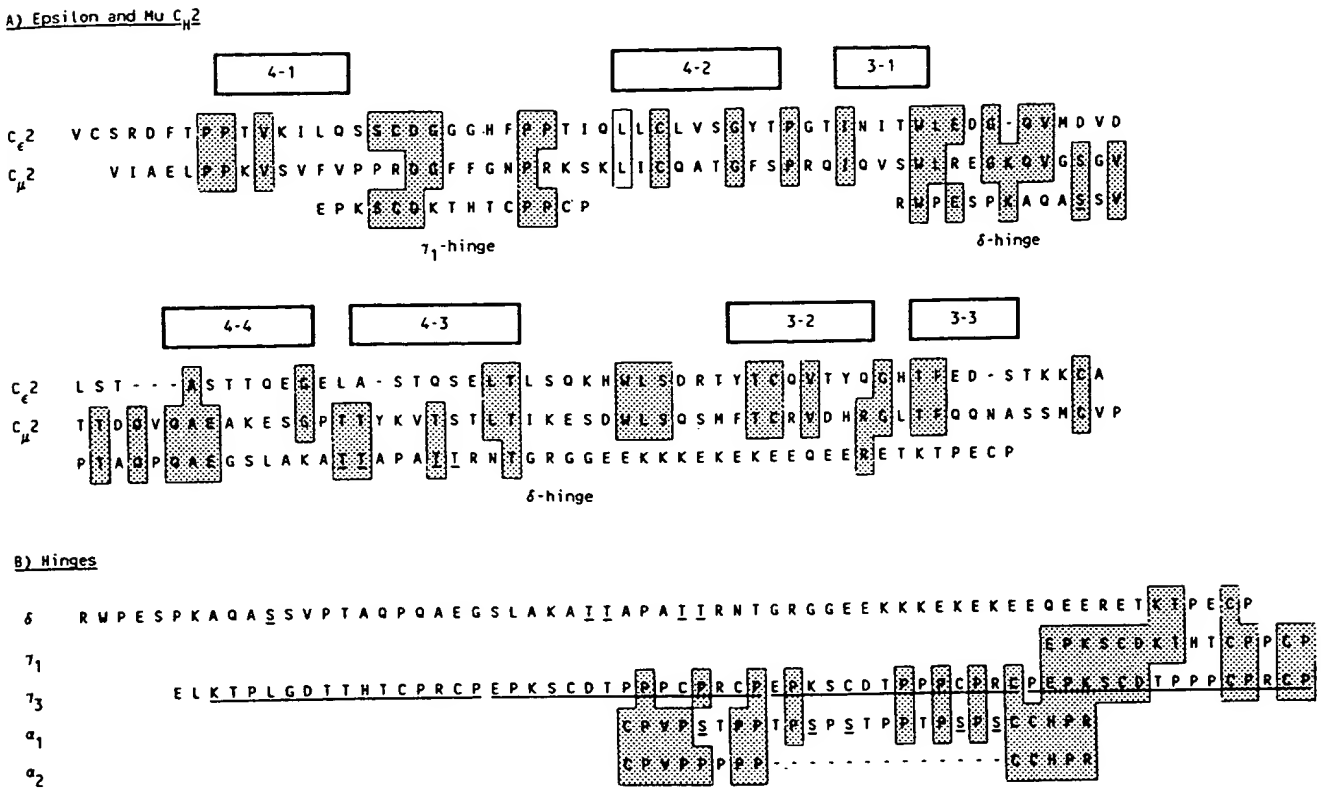


FIG. 7. Comparison of the amino acid sequences of **(A)** the $C_\mu2$ and $C_\epsilon2$ domains and **(B)** the hinge regions of the human immunoglobulins. In **(A)**, the C_H2 domains of μ and ϵ have been aligned as in Fig. 4. Also displayed is the alignment of several hinge regions from other isotypes to demonstrate the potential, although limited, relationship between hinges and the C_H2 domains. In **(B)**, several features of hinge regions are demonstrated. While homology between the hinges of different classes is limited (mostly proline and cysteine identities), the hinges of the subclasses (γ_1 and γ_3, α_1 and α_2) are quite homologous. Note the fourfold duplication of the γ_1 hinge seen in the γ_3 hinge highlighted by the *underscoring* of the duplication unit (ref. 25). Also note the deletion within the α_2 hinge compared to α_1. Threonine and serine residues involved in the glycosylation of the δ and α hinges are underscored (refs. 20 and 26).

IgE secretion have been shown to be inhibited (30); (c) resistance to proteolytic digestion—as in the α hinge region; (d) solubility of immunoglobulin in aqueous solutions is probably enhanced by carbohydrate substituents; and (e) the clearance of immunoglobulin from serum is modulated by the interaction of carbohydrate moieties with specific receptors in the liver (31).

Light Chains

Immunoglobulin light chains consist of two domains, V_L and C_L, giving a polypeptide of approximately 214 amino acids and a molecular weight of approximately 23,000. The constant region is found in two types, kappa (C_κ) and lambda (C_λ), which represent the products of separate genes located on separate chromosomes (32,33). In humans there is only one κ constant region, but there are at least four and likely six subtypes of λ, which, as in the heavy chain, represent the products of separate constant region genes. C_κ and C_λ are particularly similar, probably in an analogous fashion to the high homology of the C_H1 domains wherein the role of pairing with a heavy chain places structural constraints on the primary sequence. Figure 8 contains sequence comparisons of the κ and λ constant regions from several species. κ and λ are more highly related to one another and are more conserved between species than the heavy chains. Like the heavy chain domains, several consistent features are apparent. The invariant cysteines and tryptophan that are responsible for domain folding are conserved and occupy homologous positions in the C_L domain. The predominant conservation of amino acids located in the β strands is also consistent with the data for heavy chain domains.

The ratio of κ and λ light chains used in immunoglobulins varies from species to species. In humans the κ:λ ratio is approximately 70:30, while in the mouse it is 95:5 (12). The exact mechanisms underlying this observation are unknown. The most acceptable hypothesis to date is that the ratio of expressed light chain types correlates with the number of variable region gene segments; that is, the human is estimated to have 300 V_κ genes and 100 V_λ genes, the ratio therefore being 3, which is reasonably

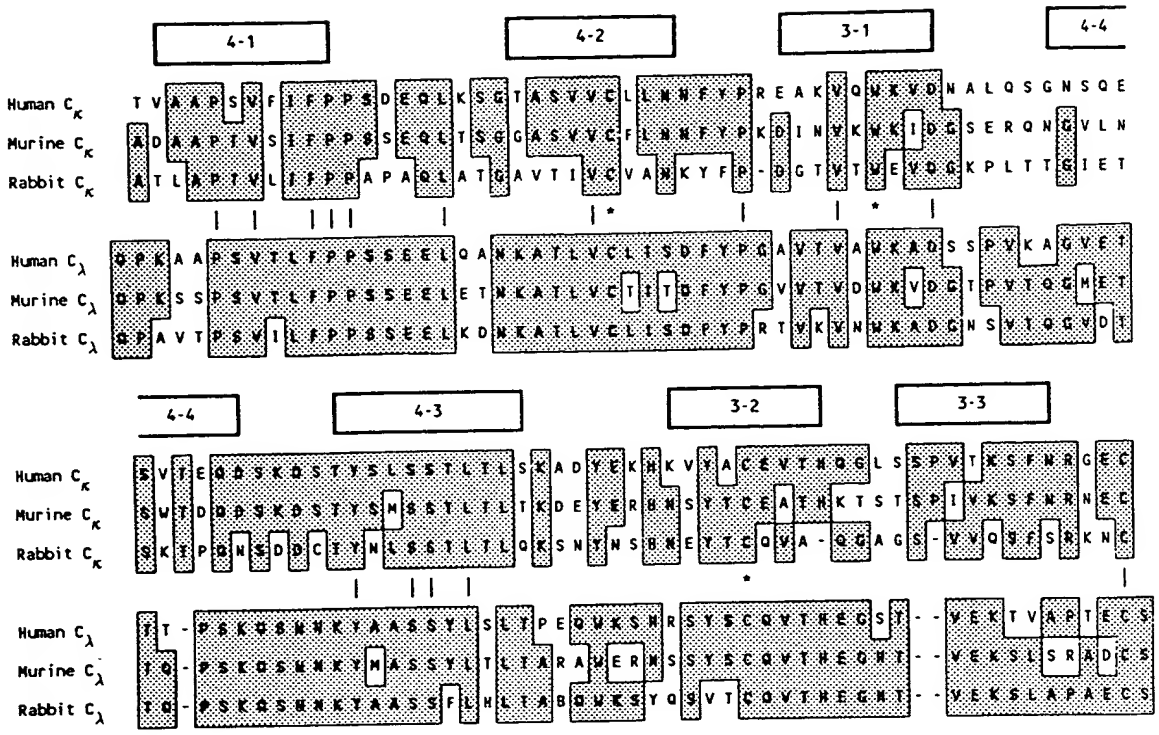


FIG. 8. Comparison of the amino acid sequences of the C_κ and C_λ domains from several species. Sequences were aligned as in Fig. 4. *Asterisks* indicate the invariant cysteine and tryptophan residues. *Vertical bars* between the C_κ and C_λ sequences indicate invariant positions between all six sequences. The most carboxy proximal cysteine in all six sequences is that which forms the H–L interchain disulfide bond.

close to the expressed κ:λ ratio. Theories based on preferential H–L pairing or differences in the regulation of gene expression have not been ruled out, however.

Variable Regions

Like constant region domains, variable regions are typical immunoglobulin domains, with all the structural features that define the immunoglobulin fold. There are two important features of the variable regions that set them apart from the constant regions. First, unlike heavy and light chain constant regions, which are encoded by at most 15 genes collectively, the number of variable region genes has been estimated to be 1,000 or greater. The second distinguishing feature of variable regions is in fact their variability. In order to express variability of each amino acid position in a quantitative fashion, Wu and Kabat (34) developed a method to determine and display variability, defined as follows: variability equals the number of amino acids at a given position divided by the frequency of the most common amino acid at that position. The denominator is the number of times the most common amino acid occurs divided by the number of proteins examined. Thus, for the example in their original study, at position 7 of light chains, 63 proteins had been sequenced and serine occurred 41 times. Four different amino acids (proline, threonine, serine, and aspartic acid) had been detected at this position. The frequency of the most common amino acid was thus $^{41}/_{63} = 0.65$ and the variability was $^{4}/_{0.65} = 6.15$. In this equation, an invariant residue would have a variability of one, whereas the theoretical upper limit for 20 amino acids occurring randomly would be $20/(^{1}/_{20}) = 400$. The variability profiles for human heavy chain V regions and the cytochrome *c*'s of various species are presented in Fig. 9. The plot for the cytochromes displays the fact that variability is a normal feature of proteins. The stretch between positions 70 and 80 is the active site of cytochrome *c*, and the plot reflects the conservation of sequence that is necessary for cytochrome activity. The variability within the human V_H sequences is distributed quite differently. The V_H variability is not uniformly distributed and reaches peak values more than three times that of the cytochromes. The areas of high variability are termed the hypervariable regions (HVR) or complementarity determining regions (CDR); the conserved areas are called the framework regions. Figure 10 shows the positions of the CDRs in several human variable domains. It is important to note that the positions of the CDRs primarily coincide with the loops between β strands, and conversely, the frameworks correlate with the β-strands themselves; that is, CDR1 lies between β strands 4-2 and 3-1, and CDR2 is between 3-1 and 4-4. Also note that, as was the case with the constant regions, several amino acids are invariant between variable regions. These invariant amino acids are those seen in the constant region domains (Cys and Trp) and are again presumed to fulfill

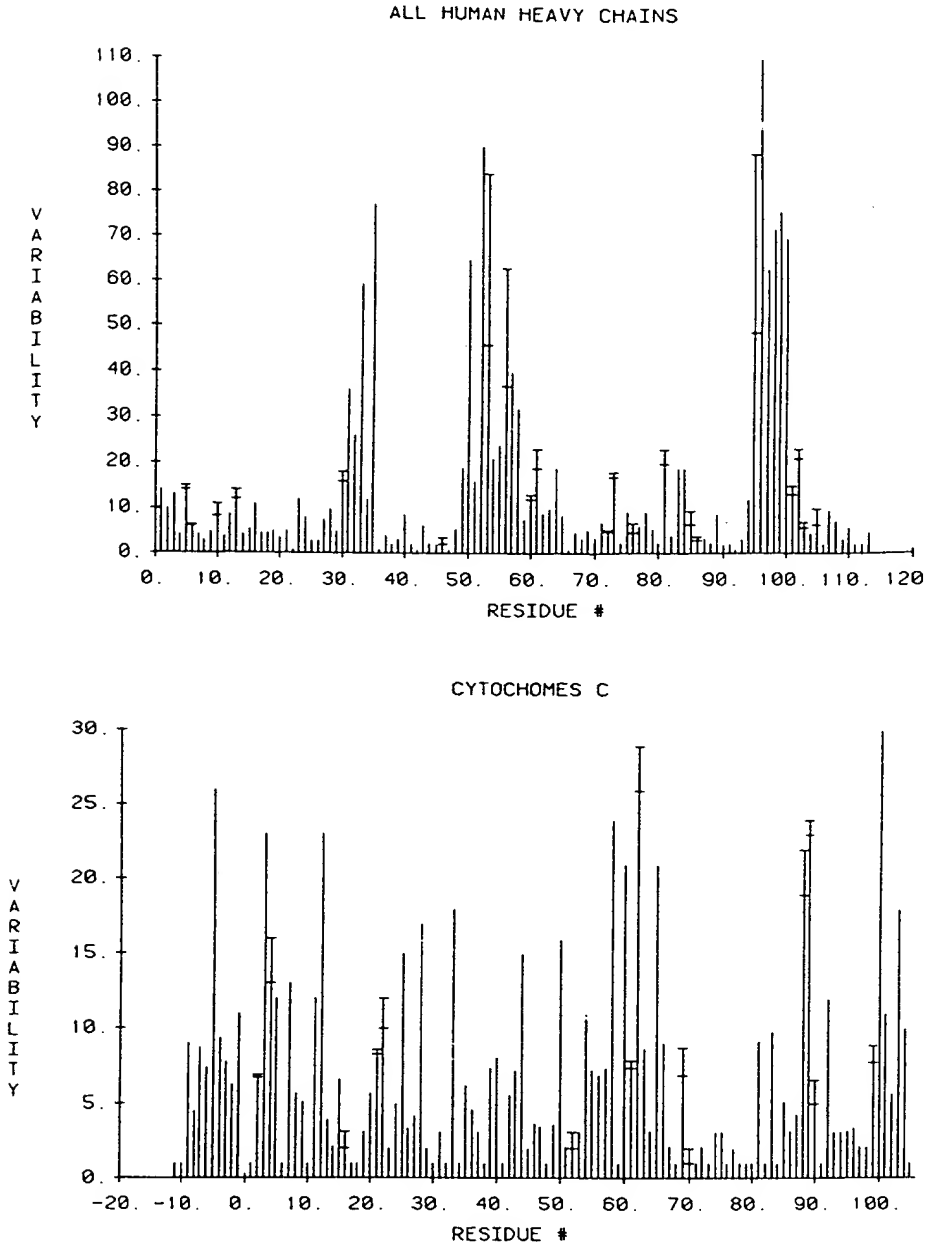


FIG. 9. Variability plots for the human heavy chains and cytochromes *c* from various species. Note that the *Y* axes are of different scales in the two plots. The hypervariable regions of the immunoglobulin heavy chains are seen as the three obvious peaks of variability in the plot. (From Kabat et al., ref. 35, with permission.)

a structural role in the immunoglobulin fold. Colorplate 3 (see page 220) shows the three-dimensional structure of F_{ab} New with the amino acids of the hypervariable regions highlighted. Recall from the earlier discussion of the immunoglobulin fold that the three-stranded β sheets of variable regions are the contact areas between the V_H and V_L regions. Also recall that this contact is somewhat loose, which is claimed to form a deep groove for antigen binding. Colorplate 3 makes this point clear, in that the hypervariable regions do in fact line this groove as well as the extended loops. This pocket has been shown by several techniques to be the antigen-combining site of the immunoglobulin molecule (review in ref. 36). This correlation of variability with the three-dimensional structure of the immunoglobulin domain is consistent in all species studied.

Closer examination of variable region sequences has revealed that V_H and V_L regions can be grouped into families. The members of a family are more like one another than members of another family. An arbitrary guideline for assigning V region families can be adopted where, for example, all V regions that bear ≥80% protein sequence homology to each other are members of the same family. Using such a guideline, the members of a family as a whole are approximately 75% identical, while members of different families are approximately 50% identical. Table 1 demonstrates the similarities between the human V_H families in tabular form, while Fig. 11 demonstrates this point with the alignment of the human V_κ and V_H region sequences. The figures in Table 1 represent the percent identities between several human V_H region sequences. The identity between V regions within a family

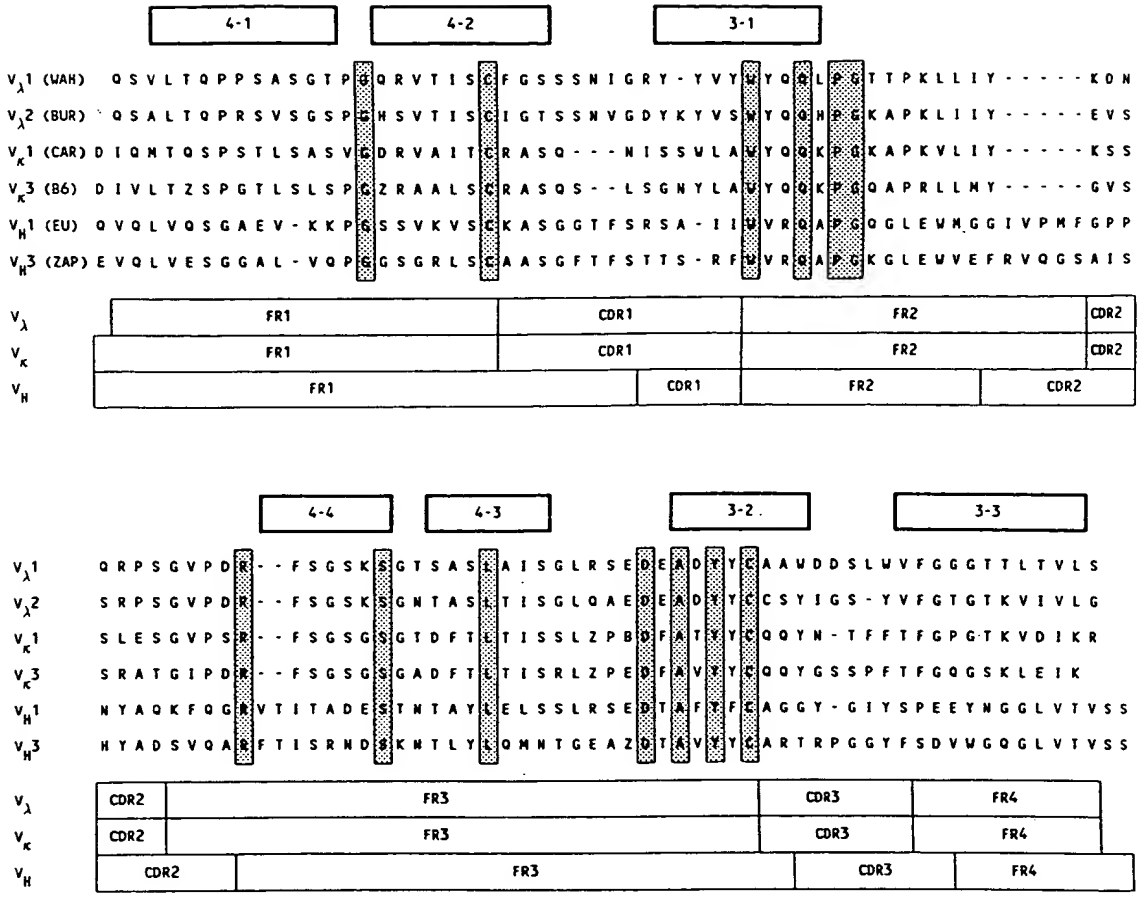


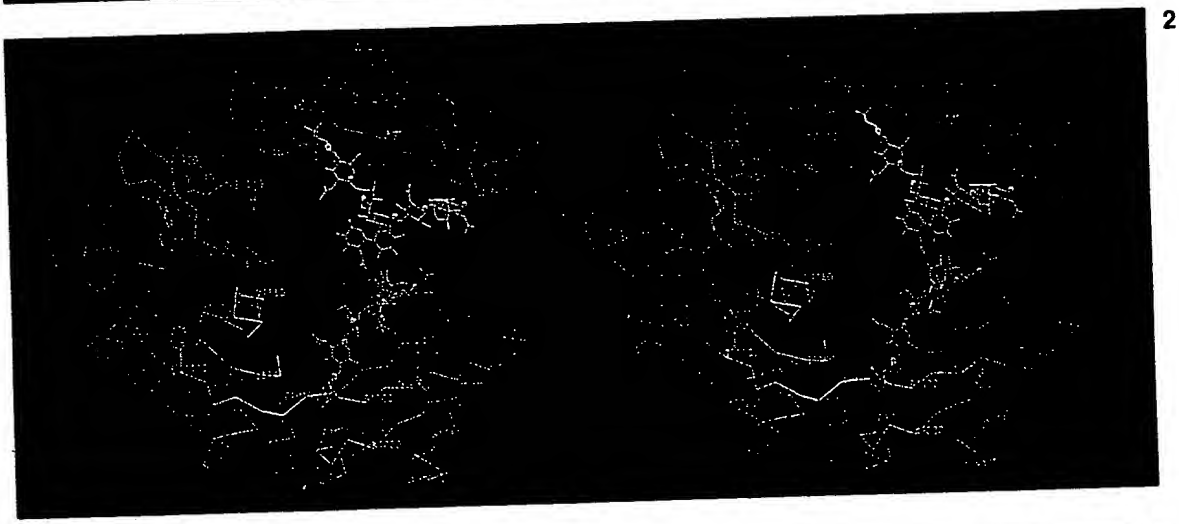
FIG. 10. Comparison of the amino acid sequences of several human variable regions. Sequences aligned as described in Fig. 4. Positions of the β strands as described in Fig. 2. Below the aligned sequences is a block diagram of the positions of the framework (FR) and complementarity-determining regions (CDRs) in the three types of variable regions. Note the different sizes of CDR1 and CDR2 between the light and heavy chain V regions.

COLORPLATE 1. Stereo pair (see note at end of chapter) of the F_{ab} portion of the human myeloma protein New. The F_{ab} is viewed such that the variable region domains are at the **top** of the figure, while the $C_\gamma 1$ and C_λ domains are at the **bottom**. The heavy chain is colored *red*, the light chain *blue*, and the cysteines of the disulfide bonds are *green*. Note the close contact between the four-stranded β-pleated sheets of $C_\gamma 1$ and C_λ, as contrasted to the somewhat more loose association of V_λ and V_H via their three-stranded β-pleated sheets. The switch region appears as a flexible joint between the two globular domains. (Atomic coordinates from Saul et al., ref. 19, with permission.)

COLORPLATE 2. Stereo pair of the F_c fragment of human pooled IgG. The heavy chains are depicted in *yellow* and *red*, the carbohydrate substituents are depicted in *blue*, cysteine residues are highlighted in *green*. Note the close pairing of the $C_\gamma 3$ domains via their four-stranded β-pleated sheets. The carbohydrate groups fill the interdomain space between the $C_\gamma 2$ domains, forcing this region to achieve a different conformation than that seen between the other immunoglobulin domains. (Atomic coordinates from Deisenhofer et al., ref. 23, with permission.)

COLORPLATE 3. Stereo pair of F_{ab} New with the amino acids of the hypervariable regions highlighted. The F_{ab} is viewed nearly end on, with the loops of the hypervariable regions pointing slightly to the left. The heavy chain is depicted in *blue*, the light chain in *red*. Cysteine residues are in *green*. The entire face of the antibody molecule is composed of hypervariable amino acids. Also note the extension of hypervariable residues into the interface between the three-stranded β-pleated sheets.

1



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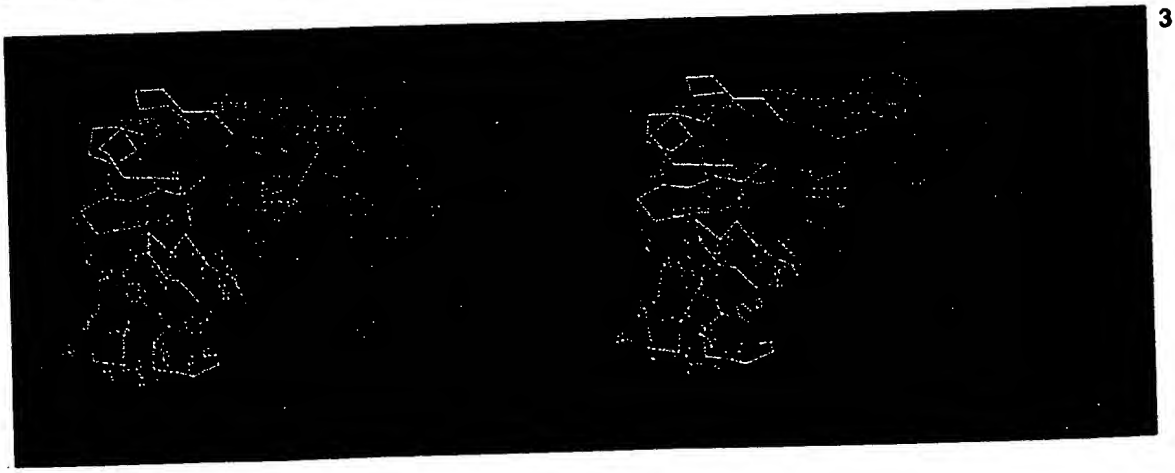

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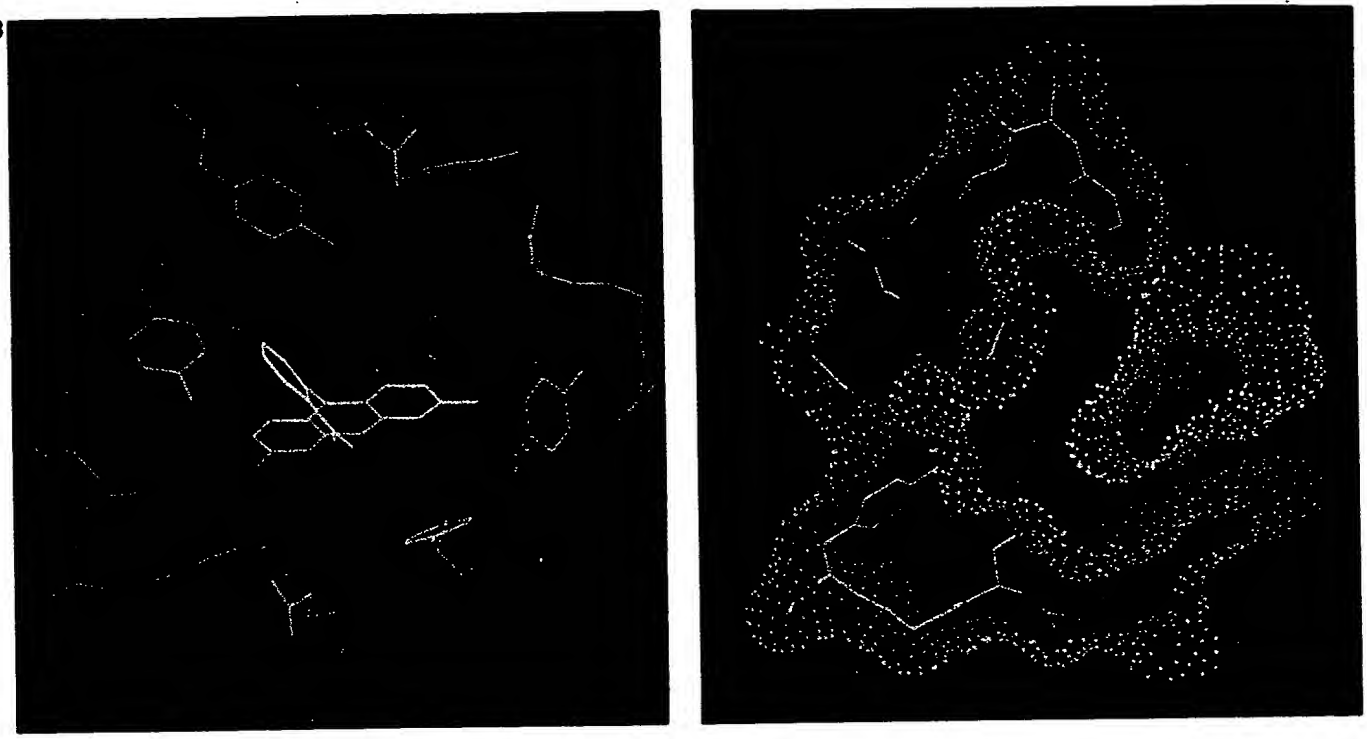
4A,B



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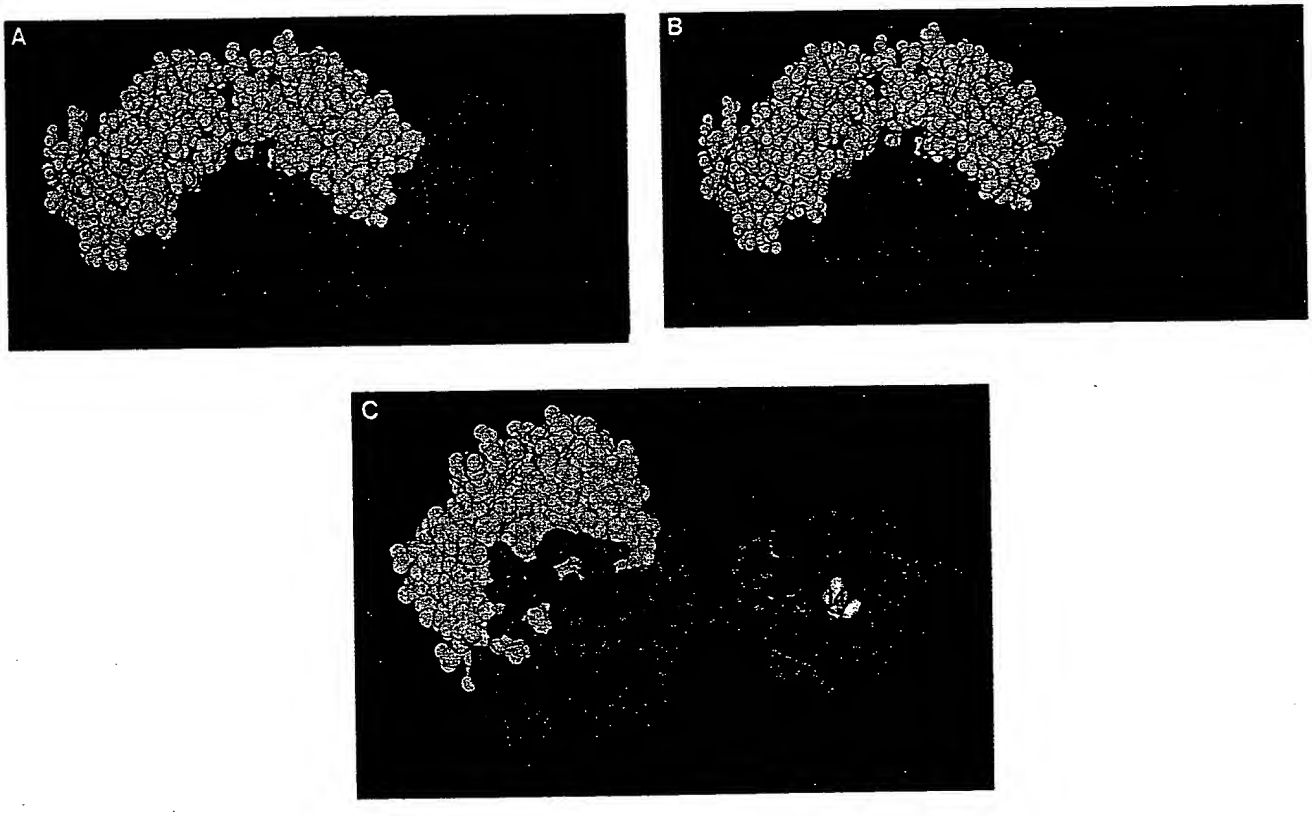

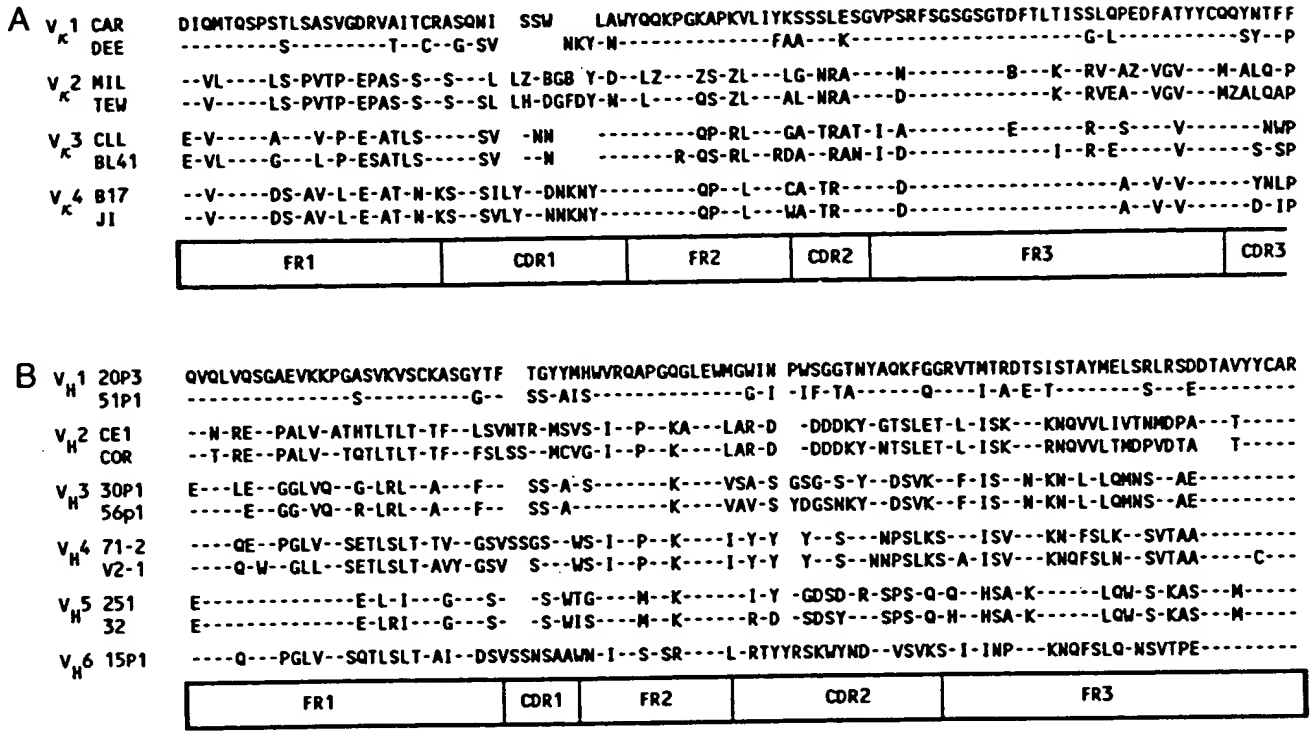

FIG. 11. Comparison of the amino acid sequences of several human **(A)** V_κ and **(B)** V_H families. Sequences aligned as described in Fig. 4. Amino acid positions identical to the reference sequence are indicated by (—); differences are indicated as the single-letter amino acid substitution. Below the aligned sequences is a block diagram of the positions of the framework (FR) and complementarity-determining regions (CDRs). The division of families is most apparent as the linked substitutions seen within a family as compared to the reference sequence.

(on the diagonal) range between 80 and 91%, while members of different families (off diagonal) are only from 32 to 68% identical. In Fig. 11 representatives of the human V_κ and V_H families have been aligned. In Fig. 11A representatives of the four human V_κ families are aligned, while Fig. 11B contains V_H sequence alignments. Roughly one-half of the positions of the V_κ sequences are identical, due to the simple fact that they are all V_κ sequences and as such derive from common progenitors. The other half of the positions have shared substitutions when compared to each other. The same can be said for the V_H sequences. Within a family several positions will share an amino acid substitution as compared to the remainder of the V region sequences. This reflects the fact that the V_κ and V_H families diverged relatively early in immunoglobulin evolution, and each family thus bears the unique sequences derived from these family progenitors. The shared substitutions seen within a family are characteristic of that family and can be used to quickly identify a particular sequence as belonging to a particular family.

COLORPLATE 4. Computer models of the interaction of the Mcg Bence-Jones dimer (*blue*) with fluorescein (*yellow*). **A**: The antibody is depicted end on (as in Fig. 3), with the amino acids of the antibody molecule, which are in close proximity, displayed and showing the pocket formed by the Mcg dimer interface. **B**: The molecules are viewed from the side, with a surface contour added to further emphasize the close fit of the two molecules. These images give a good representation of the deep pocket binding seen in small molecule–antibody interaction. (From Edmundson et al., ref. 69, with permission.)

COLORPLATE 5. Space-filling models of F_{ab} D1.3 (an anti-lysozyme antibody) and lysozyme. The heavy chain of D1.3 is depicted in *blue*, the light chain in *yellow*, the lysozyme molecule in *green*, and glutamine-121 in *red*. **A**: The model is shown as it was seen in the crystal structure, with the two molecules creating a blunt, end-on interface. **B**: The molecules have been pulled apart to demonstrate the complementarity of the two molecule surfaces. **C**: The molecules have been rotated toward the viewer to demonstrate the number of contact residues involved in the antigen–antibody interface (contacting residues now in *red*, Gln-121 in *light purple*). (From Amit et al., ref. 70, with permission.)

TABLE 1. *Comparison of the percent identity between the human V_H families*[a]

		VH1	VH2		VH3		VH4		VH5		VH6
		51P1	CE1	COR	30P1	56P1	71-2	V58	251	32	15P1
VH1	20p3	[80]	33	36	53	55	46	44	64	64	37
	51p1		32	35	57	58	48	44	63	65	41
VH2	CE1			[82]	43	46	57	50	37	39	49
	COR				48	51	59	49	41	41	52
VH3	30p1					[88]	56	51	53	56	50
	56p1						54	50	54	55	51
VH4	71-2							[86]	49	51	68
	v58								47	49	62
VH5	251									[91]	39
	32										41

[a] Values in the table are calculated as in Table 2. Note the boxed values on the diagonal, which illustrate the high homology between family members as contrasted to the off-diagonal values.

Another important point demonstrated in Fig. 11 is that the hypervariable regions of the different families are of different lengths. Thus the different V region families intrinsically have different binding site structures independent of the amino acid sequences therein.

Figure 12 is a closer look at one of the V_κ families, the V_κIII family. Note that at the positions marked with an asterisk the V_κIII sequences can be split into two groups. Shared substitutions like these can be used to further divide families into subfamilies; the differences noted for the V_κIII family above do in fact split it into the subfamilies $V_\kappa III_a$ and $V_\kappa III_b$. The criteria for establishing subfamilies are less well defined than for establishing families. Depending on the particular feature of immunoglobulin structure being investigated by different laboratories, the designation of subfamilies can be somewhat overlapping. The existence of families and subfamilies is a consequence of the genetic duplications of ancestral genes. Thus families are the product of early gene duplications, while subfamilies represent more recent duplications. This is an analogous situation to the classes and subclasses of the constant region genes.

Table 2 is a summary of the known V_H and V_L families for humans and mice. Where it is applicable, similar families between species have been indicated. The functional significance of V region families is discussed later.

Accessory Molecules

J Chain

J chain is a 137 amino acid polypeptide which is involved in the polymerization of IgA into dimers (sometimes trimers or more) and IgM into pentamers (37). J chain has a high content of negatively charged amino acids as well as 8 cysteine residues involved in both intrachain and interchain disulfide bonds. The human J chain gene has been cloned and sequenced (38), as has a cDNA of the murine J chain (39). These clones have been used as probes to determine that there is only one J chain gene in the human and murine genomes, and that these genes are not linked to any of the loci encoding immunoglobulins. The sequence of J chain is not significantly related to the sequence of an immunoglobulin domain; however, predictions of its secondary structure would indicate that it has a β-barrel structure very much like that of the

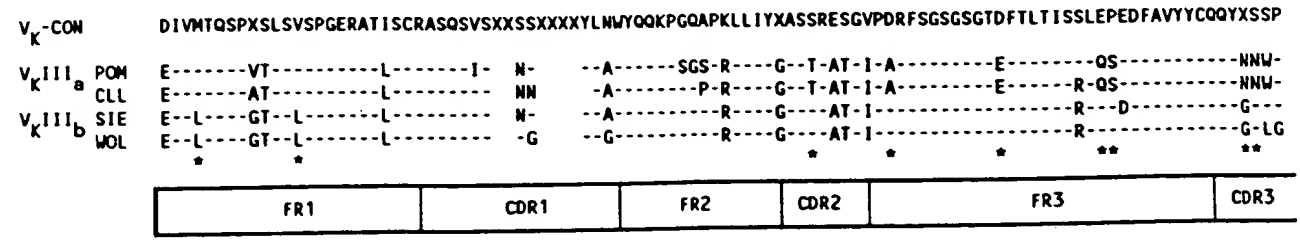

FIG. 12. Comparison of the amino acid sequences of four V_κIII molecules, representing the $V_\kappa III_a$ and $V_\kappa III_b$ subfamilies. Sequences aligned as described in Fig. 4. Amino acid positions identical to the reference sequence (V_κ = Con) are indicated by (—); differences are indicated as the single-letter amino acid substitution. Below the aligned sequences is a block diagram of the positions of the framework (FR) and complementarity-determining regions (CDRs). The reference sequence in this figure is a consensus sequence for the κ variable regions of human immunoglobulins. Positions marked with an *asterisk* are characteristic of the differences between the subfamilies.

TABLE 2. *Summary of the human and murine V_H families and their relation to one another*[a]

Human		Murine		
		Representative sequence	Nomenclature New	Old
V_H1	(74%)	J558	V_H1	V_H2
V_H2	(71%)	3609	V_H8	
V_H3	(74%)	S107	V_H7	V_H3
		J606	V_H6	
		7183	V_H5	
		X24	V_H4	
V_H4	(76%)	36–60	V_H3	V_H1
V_H5		Q52	V_H2	
V_H6		V_{gam}	V_H9	

[a] The murine V_H families are known by several names, either by the name of a representative sequence, or by the old (Wu and Kabat, ref. 35) or new (Rathbun et al., ref. 67) V_H nomenclature. Percent identities are estimations based on the average identity between several members of each family.

immunoglobulin fold (40). Expression of the J chain polypeptide is coordinately regulated with immunoglobulin expression. Unstimulated B cells contain little or no J chain, while mitogen stimulation causes a dramatic increase in J chain mRNA and protein levels (41).

The stoichiometry of IgM and IgA polymerization is such that only one J chain is associated with each IgA dimer or IgM pentamer. The exact mechanism by which J chain is involved in polymerization of IgA or IgM is unknown, but several models have been proposed (42,43). Two models for the formation of an IgA dimer or IgM pentamer are presented in Fig. 13. In the first model, the tail segments of two monomers are attached to J chain. For IgA this is the terminal stage, while for IgM, some difference in the tail or $C_\mu4$ allows the polymerization to proceed further. In this model of pentameric IgM, the tails of all the monomer units are involved in the interconnection of the pentamer. This model does not have an obvious reliance on the J chain to initiate the process. In the second model, J chain initiates the process by forming a disulfide bond with the penultimate cysteine in a single monomer. This activated monomer then interacts with another monomer's tail. Through a series of similar disulfide exchanges, the final pentameric form is achieved. In either model, details of the mechanisms by which IgM polymerization stops at five and IgA polymerization stops at two are unknown. One possible explanation is that an enzyme is responsible for the catalysis and specificity of multimer formation. Such an enzyme has been reported from an IgM-producing cell line (44).

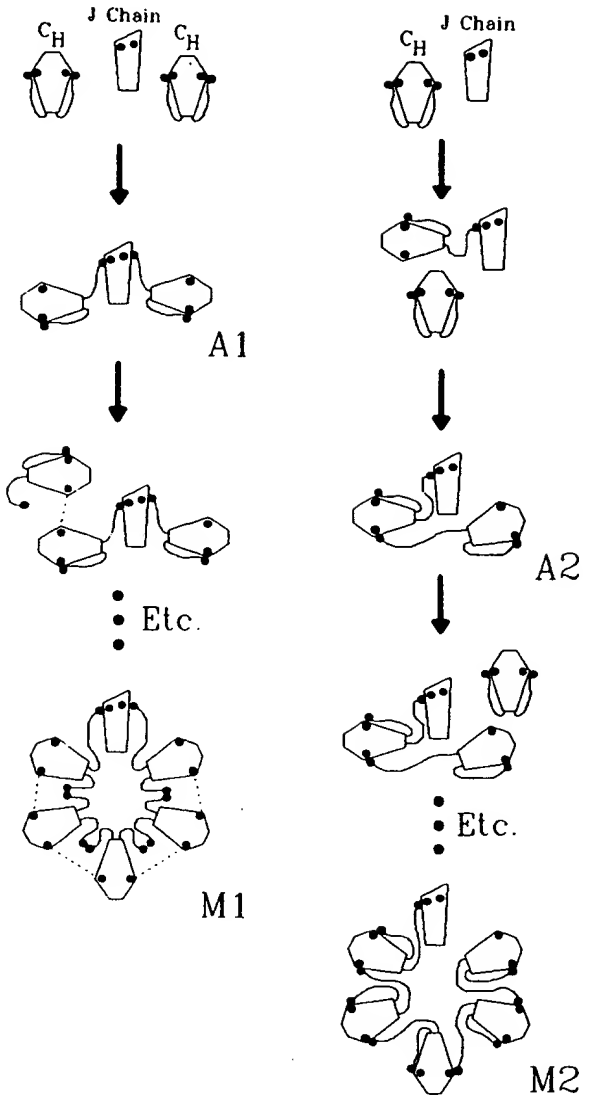

FIG. 13. Two models for the formation of pentamers of IgM and/or dimers of IgA. The model on the **left** represents the more accepted view of multimer formation, proceeding to IgA dimers (A1) or IgM pentamers (M1). The model on the **right** represents a proposal for a process more dependent on J chain to initiate the process. Details of each model are presented in text. (From Pumphrey, ref. 43, with permission.)

Secretory Component/Poly-Ig Receptor

Secretory component (SC) was initially reported as a polypeptide either covalently or noncovalently (depending on species) associated with the IgA found in external secretions. It was also found as a free chain in mammary and parotid secretions. Recently, it was determined that SC is the binding domain of a transmembrane receptor for poly-Ig complexes [$(IgM)_5$, $(IgA)_2$] (45). The entire molecule is called the poly-Ig receptor (poly-IgR). Poly-IgR is synthesized in many types of glandular epithelia, where it is expressed on the basal membrane of the epithelial cells. The entire nucleotide sequence of the poly-IgR has been determined from a rabbit poly-IgR cDNA. Interestingly, the poly-IgR molecule is a member of the immunoglobulin superfamily (46). It consists of six immunoglobulin-like domains and a seventh unrelated do-

main. The amino proximal five of these domains are extracellular and predicted to have tertiary structures very similar to the immunoglobulin fold. The sixth domain is also like an immunoglobulin subunit at its amino end, but the homology ends in an area that is predicted to be the transmembrane stretch of the polypeptide. The carboxyl domain is an intracellular domain of 103 primarily hydrophilic amino acids. The poly-IgR functions as a specific receptor for poly-Ig molecules at the basolateral surface of epithelial cells. The poly-IgR–poly-Ig complex is endocytosed via coated pits and transported in intracellular vesicles to the apical cell surface, where the complex is exocytosed. During its movement through the cell, the receptor molecule is cleaved into two subunits—SC as previously described and the membrane-associated carboxy terminus. Thus the poly-IgR is a one-time receptor, performing its transport function once and then degrading. The exact site within the polypeptide chain where receptor cleavage takes place is unknown, although it has been localized to the extracellular region of the sixth Ig-like membrane-spanning domain.

Immunoglobulin Synthesis and Secretion

The genetic events that generate and regulate the transcriptional units of immunoglobulin heavy and light chains are complex. The specific details of immunoglobulin gene rearrangement and the regulation of gene expression are covered in Chapter 10. Given that an active mRNA is being transcribed, however, that mRNA must still undergo the processes of translation and intracellular transport in order to produce a functional antibody molecule. The details of heavy and light chain synthesis are like those for most secretory glycoproteins and are reviewed in depth elsewhere (47,48). Both heavy chains and light chains have a signal peptide of 16 to 26 amino acids that directs the ribosome to the rough endoplasmic reticulum (RER). The membrane form of heavy chains is left inserted in the RER membrane, while both light chains and the secreted form of heavy chains are secreted into the cisterna of the RER. During the synthesis and extrusion of the nascent polypeptide through the RER membrane, the signal peptide is removed and core oligosaccharides are attached (49). The subsequent trafficking of these polypeptides through the Golgi, where carbohydrate modifications are made, and ultimate exocytosis at the cell surface are beyond the scope of this chapter.

One aspect of immunoglobulin synthesis is noteworthy, however. A 78-kd protein is found associated with free heavy chains in the RER (50,51). This protein, called BiP (*i*mmunoglobulin heavy chain *b*inding *p*rotein), appears to regulate the post-translational fate of heavy chains. As is discussed in Chapter 14, B cells go through several stages of differentiation before they become antibody-secreting cells. A pre-B cell produces only cytoplasmic μ chains. These μ chains are found in a one-to-one association with BiP in the RER and are never secreted. When a light chain is produced, signaling the differentiation to an immature B cell, BiP is found to associate only with incompletely assembled immunoglobulin molecules (i.e., H, H_2, HL, H_2L). The implication is that BiP and light chains are in competition for binding to heavy chains. In normal B cells incomplete immunoglobulins are never secreted. These facts taken together imply that BiP functions to retain incomplete immunoglobulins in the RER. There is a significant body of data to support this conclusion. For example, in B cells that produce mutant heavy chains wherein the C_H1 domain is either missing or mutant, the various forms of incomplete immunoglobulin molecules are secreted. In these same cells, although BiP is synthesized, it is never found associated with the heavy chains in the RER. This also implicates the C_H1 domain as the region of association between BiP and heavy chains.

Whole Immunoglobulins

The differences in primary structure pointed out in the preceding sections are reflected in the quaternary structures of the various immunoglobulin classes. Figure 14 is a collection of two-dimensional representations of the covalent structures of the human immunoglobulins. The predominant differences between these structures are the number and placement of interchain disulfide bonds and carbohydrate substituents. The disulfide bonds formed in immunoglobulin molecules are generally very highly conserved. The intradomain disulfide bonds are practically invariant between domains, classes, and species. The disulfide bond, which attaches heavy and light chains, is also highly conserved. In the human immunoglobulins the half cystines that comprise this bond are located near the amino end of C_H1 and near the carboxyl end of C_κ or C_λ. Human IgG1 is an exception wherein the half cystine donated by the heavy chain is found near the carboxyl end of C_H1 (52). Although it is not shown in this figure, IgA of the A2m(1) allotype has L–L and H–H disulfide pairing rather than the usual L–H pairing (53). These variations are structurally possible because the half cystines involved are close in space in the three-dimensional structure. The disulfide bonds between heavy chains are the most variable in number and position and in many cases are not even formally proved to exist as depicted in Fig. 14. Generally, the H–H bonds are formed between the hinge regions, or in the case of μ and ε, analogous positions in C_H2. Both μ and α also have an extra cysteine very near the carboxy terminus which is involved in the disulfide bond formed in the J-chain-mediated polymerization of these classes. μ and α also have additional half cystines in the $C_\mu3$ and $C_\alpha2$ domains which are involved in the intersubunit bonds in the polymeric forms of these molecules. The bonds formed by these half cystines in the monomeric forms of IgA and IgM are either interchain (μ and α) or intrachain (α) bonds.

IMMUNOGLOBULIN FUNCTION

The immune system acts to identify and remove "foreign" agents. The role of the immunoglobulin molecu

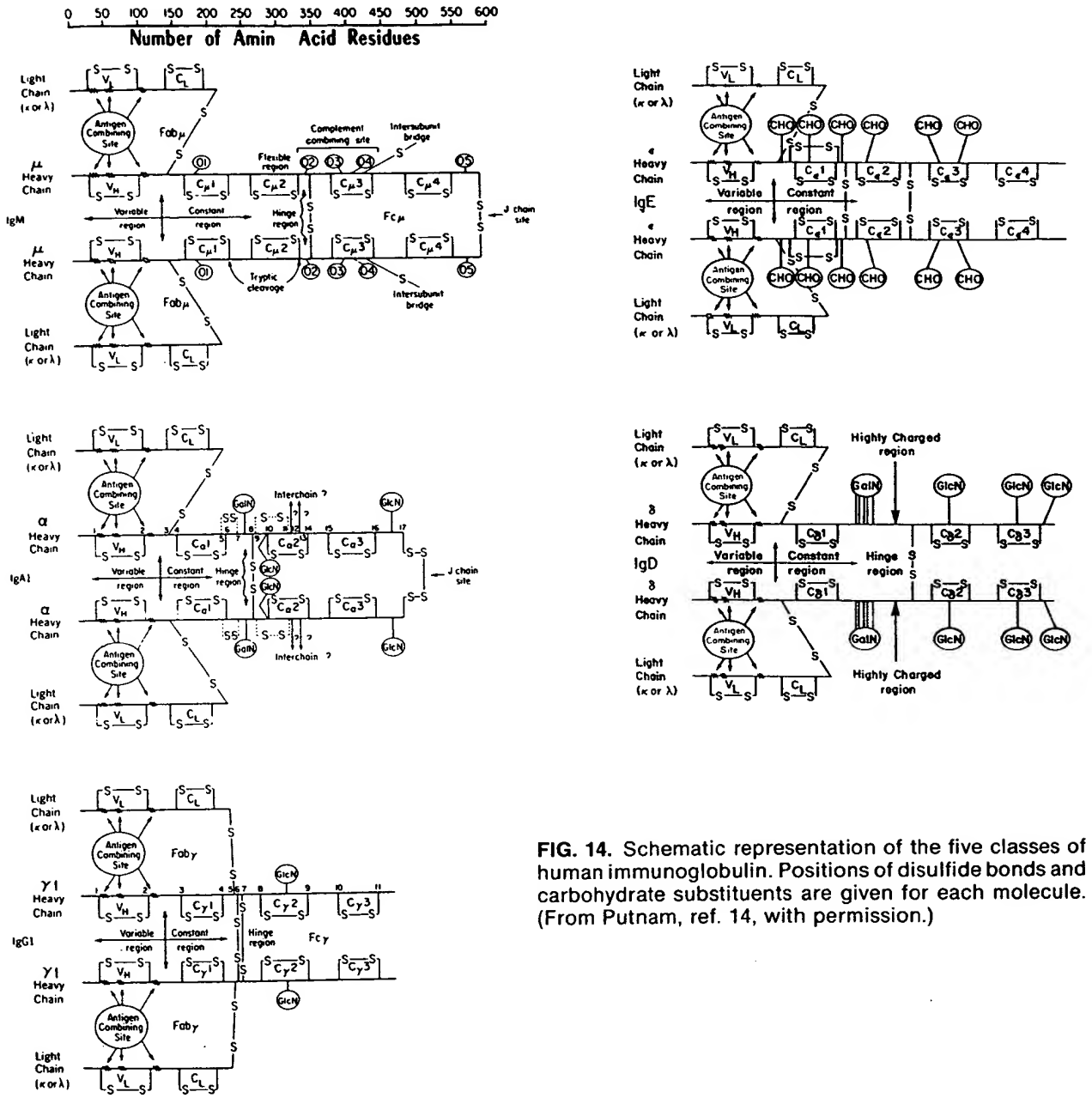

FIG. 14. Schematic representation of the five classes of human immunoglobulin. Positions of disulfide bonds and carbohydrate substituents are given for each molecule. (From Putnam, ref. 14, with permission.)

in the immune system is to provide a means for linking the recognition of "foreign" to the mechanisms that can act on the "foreign" agent. Thus the activity of immunoglobulin molecules can be divided into two separate functions: recognition and effect. Variable regions are the portions of an immunoglobulin that recognize (bind) antigenic determinants, while constant regions interact with other molecules and/or cells to accomplish the effector functions which are broadly termed humoral immunity. As was indicated in the preceding sections, the structures of the variable and constant regions are distinct, as are the structures of the different heavy chain classes. The following sections present the functional differences between the immunoglobulin classes, which must be a direct consequence of this structural heterogeneity.

Constant-Region-Associated Effector Functions

All the known effector processes mediated by immunoglobulin molecules can be ascribed to the F_c region of the molecule. This is understood as a consequence of the ability to proteolytically cleave the molecule into two fractions (F_{ab} and F_c). In all cases studied, the effector functions are associated with the F_c fragment. F_c-me-

diated effector functions can be divided into three general categories: (a) activation of the classical complement (C) cascade, (b) interaction with effector cells, or (c) compartmentalization of immunoglubulins. In addition, the stability of the different immunoglobulin classes *in vivo* is significantly different and may play a role in the intricate regulation of the immune response. The division of these capacities among the human immunoglobulin classes is discussed separately in the following sections. Table 3 summarizes the F_c-mediated effector functions for human immunoglobulins. The precise structural features responsible for the expression of these effector functions are generally unknown, but where such information is available it is presented. The summary of isotype functions to follow relates specifically to human immunoglobulins.

IgM

IgM is the predominant antibody in a primary immune response. It is primarily found in one of two forms: membrane-bound monomeric IgM (mIgM) or secreted pentameric IgM (sIgM). As a membrane-bound receptor molecule on the surface of mature B cells, mIgM (in concert

TABLE 3. *Physical, chemical, and biological properties of human immunoglobulin classes*[a]

Property	IgG	IgA	IgM	IgD	IgE
Usual molecular form	Monomer	Monomer, dimer, etc.	Pentamer	Monomer	Monomer
Molecular formula	$\kappa_2\gamma_2$ or $\lambda_2\gamma_2$	$(\kappa_2\alpha_2)_n$ or $(\lambda_2\alpha_2)_n$	$(\kappa_2\mu_2)_5$ or $(\lambda_2\mu_2)_5$	$\kappa_2\delta_2$ or $\lambda_2\delta_2$	$\kappa_2\epsilon_2$ or $\lambda_2\epsilon_2$
Other chains	—	J chain, S piece	J chain	—	—
Subclasses	IgG1, IgG2, IgG3, IgG4	IgA1, IgA2	None established	None	None
Subclass heavy chains	$\gamma1$, $\gamma2$, $\gamma3$, $\gamma4$	$\alpha1$, $\alpha2$	—	—	—
Heavy-chain allotypes	Gm (ca. 20)	Am (2)	Mm (2)	—	—
Molecular weight	150,000	160,000	950,000	175,000	190,000
Sedimentation constant (Sw^{20})	6.6S	7S, 9S, 11S, 14S	19S	7S	8S
Carbohydrate content (%)	3	7	10	9	13
Serum level (mg/100 ml) (adult average)	1250 ± 300	210 ± 50	125 ± 50	4	0.03
Percentage of total serum immunoglobulin	75–85	7–15	5–10	0.3	0.003
Total circulating pool (mg/kg of body weight)	494.0	95.0	37.0	1.1	0.019
Half-life (days)	23.0	5.8	5.1	2.8	2.5
Rate of synthesis (mg/kg of body weight per day)	33.0	24.0	6.7	0.4	0.016
Paraproteinemia	Myeloma	Myeloma	Macroglobulinemia	Myeloma	Myeloma
Antibody valence	2	2	5 or 10	?	?
Complement fixation (classic pathway)	+(IgG1, 2, 3)	—	+	—	—
Complement activation (alternative pathway)	+(IgG4)	+(IgA1, 2)	—	+	—
Binding to cells	Macrophages, neutrophils	—	—	?	Mast cells
Other biological properties	Secondary Ab response; placental transfer	Characteristic Ab in mucous secretions	Primary Ab response; rheumatoid factor	Main lymphocyte cell surface molecule	Homocytotropic Ab anaphylaxis; allergy

[a] Data on biosynthesis, turnover rate, and circulating pool from Waldman et al. (54), with permission.

with other factors) acts to signal the activation, proliferation, and differentiation of the mature B cell. sIgM acts as the first defense in humoral immunity, appearing early in the immune response. The sIgM of the primary immune response has not undergone the process of affinity maturation, therefore the specific affinity of IgM for its antigen is relatively low. The fact that sIgM is a pentamer allows for multiple Ab–Ag interactions to occur when the Ag is polyvalent (as is the case for most bacterial and viral surface antigens). Thus, in its secreted form, IgM pentamers can bind antigen with a high avidity. Ab–Ag complexes of this type activate the classical complement cascade quite efficiently. Additionally, it has been observed that Ab–Ag–C complexes are phagocytosed by macrophages. This phagocytosis is probably mediated by C3b receptors on the macrophage and not by a receptor specific for the IgM F_c region.

IgM is also the antibody most frequently observed in autoimmune disorders (i.e., rheumatoid factor). This correlation is probably a consequence of a dysfunction in the regulation of the differentiation of an autoimmune B cell, and not a direct autoimmune function of the μ chain itself.

IgD

There is surprisingly little data available on the function of IgD. It is present as a surface receptor on mature B cells (sIgD). The ratio of expression of μ and δ can be used as an indication of the differentiation state of B cells. The IgM and IgD coexpressed on the surface of the mature B cell bear the same variable region. The redundancy of the coexpression of sIgD and sIgM, with identical receptors for antigen, confuses the exact role that either isotype plays in B cell activation. The half-life of IgD is extremely short and serum levels of IgD are practically negligible. This instability may be a consequence of IgDs extremely long hinge region.

IgG

In contrast to IgD, there is a large body of data on the function of IgG (reviewed in ref. 8). IgG makes up 75% of serum immunoglobulin. It is the predominant antibody in a secondary immune response and as such is the "workhorse" of the immune response. All four IgG subclasses can activate the classical complement cascade, although with different efficiencies. The site of interaction between complement component C1q and IgG has been mapped to the C_H2 domain, with contradictory results as to the exact amino acids involved.

There are specific receptors for the F_c regions of all four subclasses of IgG on a variety of cell types (9). F_c receptors for IgG have been demonstrated on macrophages, mast cells, polymorphonuclear cells, and lymphocytes. Interactions with these receptors mediate several functions including phagocytosis (55), the release of mediators (56,57), ADCC (Antibody Dependent Cellular Cytotoxicity) and the specific upregulation or downregulation of lymphocyte function (58). In addition, IgG is the only antibody able to cross the placenta. This ability is mediated by a placental receptor specific for the F_c of IgG (59). The interactions between IgG and its specific F_c receptors are thought to take place at the junction between $C_\gamma2$ and $C_\gamma3$ (see IgE below).

IgA

IgA is the most abundant antibody in secretions (i.e., tears, saliva, gut, milk, nasal mucus) although it also comprises 10 to 15% of the circulating immunoglobulins. IgA is the first line of defense against organisms that would invade via the mucosa and as such plays an important role in immune defense. The presence of large amounts of IgA in colostrum has been used as an argument for the transfer of maternal immunity to the neonate (review in ref. 60). The exact mechanism by which IgA affects the neonatal immune system is not known. Presuming that sIgA can survive the low pH of the stomach, it may provide the infant with gut immunity based on the immune repertoire of the mother. This and other theories are the focus of current research.

As was indicated above, the poly-IgR acts as a specific receptor for IgA at the epithelial surfaces of secretory organs. The specific site of interaction between poly-IgR and IgA has been mapped to $C_\alpha2$, where a disulfide bond is formed between poly-IgR and the half cystine found in this domain.

IgE

IgE is found in human serum at levels of less than $^1/_{100}$th of a percent of total circulating immunoglobulin. In atopic individuals, however, increased amounts of IgE are synthesized and subsequently found on the surface of mast cells and basophils. There is a high-affinity receptor for the F_c region of IgE found on these two cell types, and it would appear that the principal function of IgE is to arm these cells with specific receptors (review in ref. 9). These cells then act as the mediators of allergy and parasitic immunity by releasing potent mediators of inflammation and chemoattractants for various cell types. The details of parasitic immunity (see Chapter 35), allergy (see Chapter 32), and inflammation (see Chapter 26) can be found elsewhere in this text.

The interaction between IgE and its high-affinity F_c receptor is the best characterized Ig–F_c interaction. Experiments with synthetic peptides as inhibitors of the specific interaction between IgE and its F_c receptor have implicated a stretch of 76 amino acids between $C_\epsilon2$ and $C_\epsilon3$ as the site of F_c receptor binding (61). The high-affinity receptor from mast cells has been cloned and, like the poly-IgR, is itself a member of the immunoglobulin gene superfamily (62). The details of the interaction between these two immunoglobulin domains will be interesting, as it may provide a general model for the

intermolecular interaction between immunoglobulin-like molecules.

Variable Region Functions

The immunoglobulin variable region is an antigen specific receptor. As a receptor, it has the physical properties that any other receptor can be described to have, for example, binding constants and on/off rates. In terms of its binding properties, each antibody molecule is unique. One antibody may recognize any number of antigens via either the same or even a different epitope. Even antibodies with the same epitopic specificity may have vastly different affinities for that epitope. It is thought that a given organism's repertoire of antibody molecules is capable of interacting with *any* antigen. The question then becomes: How does an organism generate antibodies of *every* specificity, and what parameters of variable region structure affect the interactions that antibody has with the spectrum of possible antigens?

Combining Sites

The antibody-combining site is largely made up of the hypervariable regions of the heavy and light chain variable regions (review in ref. 63). While the hypervariable regions certainly provide the majority of variability in combining site structure, this does not rule out the framework regions from having some role in antigen binding. The generation of combining site diversity is accomplished in four ways: (a) the pairing of different V_H and V_L domains, (b) combinatorial diversity involving the selection of alternate V region gene segments (V,D,J) to create an active V_H or V_L gene segment, (c) junctional diversity, the generation of novel amino acid sequences at the junction of V region segments, and (d) somatic mutation, the accumulation of coding sequence variation via the random mutation of a V region gene. The latter three of these mechanisms involve the manipulation and maintenance of the DNA encoding variable region genes and are discussed in Chapter 10.

The important result of these mechanisms is the generation of diversity beyond that directly encoded in the germline repertoire of the organism. This variability is primarily focused in the hypervariable regions, especially in the case of V-(D)-J recombination, which essentially generates the entire third hypervariable region of both heavy and light chains. The process of somatic mutation operates throughout the V region and persists through all stages of B cell differentiation, coming to an abrupt halt at the plasma cell stage. The accumulation of point mutations in the hypervariable regions has an effect on the specificity and affinity of the combining site. The selection of B cell clones with ever higher affinity for antigen during a specific immune response is known as "affinity maturation" and is an important feature of the function of immunoglobulin molecules. Thus several complex genetic events underlie the generation of diversity seen in antibody-combining sites and also help to fine tune the immune response.

The pairing of heavy and light chain polypeptides to form a functional combining site is a different matter. This process is subject to the constraint that the two polypeptides retain an affinity for one another sufficient for the formation of a stable bimolecular combining site. It has been shown that heavy and light chains from different individuals, as well as from different species, have the capacity to form stable H–L pairs. This implies that some basic structural features of these polypeptides have been conserved through evolution, as was pointed out in the section on the immunoglobulin fold. However, it has been estimated that 50% of the contact amino acids involved in V_H–V_L pairing are located in the hypervariable regions, suggesting that the affinity of H–L pairing should also be variable. It would then follow that some H–L pairs would be less stable due to steric or ionic forces in the contact regions comprised of these hypervariable residues (64). Early studies with human myeloma proteins seem to substantiate this theory. In experiments involving the mixing of heavy and light chains and the subsequent free association of these polypeptides, homologous H–L pairings always showed a higher affinity of pairing than did heterologous pairs (65). This result was interpreted to imply that the *in vivo* repertoire consisted only of these "high-affinity" H–L pairs, which would reduce the available repertoire to only approximately 10% of the potential repertoire. Recently, a study utilizing murine hybridoma and myeloma proteins contradicts these early findings (66). Several examples of heterologous H–L pairs with higher affinities for one another than in the homologous pairings are given, and it was further demonstrated that even "low-affinity" H–L pairs can be found with high-affinity antigen-combining sites. These contradictory findings indicate that the precise conditions which dictate the pairing of certain heavy and light chains into functional antibody-combining sites are not yet certain. Thus the extent to which H–L pairing plays a role in the generation of diversity of antibody-combining sites is still in question.

The selection of V_H and/or V_L segments also has a profound effect on the nature of the combining site. As was indicated earlier, each family of variable regions has a characteristic length and amino acid sequence. Thus the response to a given antigen might be composed primarily of one or more particular variable region families. This has been well documented in the mouse in several antigen systems, wherein the immune response of one or more strains to very simple antigens has been shown to produce a very restricted response in terms of V region family utilization (67). In the human, there is a growing body of data to indicate that certain V region families might in fact be "autoimmune families" as evidenced by their disproportionate use in autoantibodies found in clinical autoimmune disorders (68). These data might indicate that the maintenance of separate V region families is not merely a matter of circumstance, but rather a strategy for the regulation of the nature of the humoral immune response.

Antigen–Antibody Complexes

The inference that the antibody-combining site is composed of the hypervariable regions is based on a wealth of sequence data combined with the structural and functional data of many laboratories. Until crystallographic data of Ag–Ab complexes became available, however, it was merely inference. In the last few years several Ag–Ab complexes have been crystallized and analyzed by X-ray diffraction. The structures so derived have proved both conclusive and compelling. Two distinct modes of antigen binding have been described. In hapten systems, where the epitope recognized by the antibody molecule is very small, a binding mode is observed wherein the hapten is buried in a deep pocket formed by the heavy and light chain hypervariable regions. Colorplate 4 (see page 221) is a pair of computer images of one such interaction, where a fluorescein molecule is shown buried deep in the antigen-combining site of the Bence-Jones dimer Mcg (69). Note that many of the amino acids in the hypervariable regions are in close proximity to the fluorescein molecule. It is the sum of the energy of each of these interactions which determines an antibody's affinity. It is then apparent how amino acid substitutions in the hypervariable regions can change both the physical shape and chemical nature of the combining site. Most of the early structures of Ab–ligand interactions were of this type, and it was thought that most binding interactions would involve this mode of deep binding.

Recently, two crystal structures of Ag–Ab complexes involving protein antigens have been solved. Colorplate 5 (see page 221) is a model derived from the crystal structure of an antibody–lysozyme complex (70). The mode of binding in this interaction involves the entire antibody-combining site. All six hypervariable regions are involved in the interaction. The two proteins form an interface, which can be described as two flat surfaces with complementary depressions and protrusions. The structure of another protein–antibody interaction (influenza virus neuraminidase) is very similar in its general features (71). These models are the embodiment of the lock-and-key model of Ag–Ab interactions proposed decades ago. In the lysozyme Ag–Ab complex, Gln-121 of the lysozyme molecule is especially interesting. It protrudes into the cleft between V_H and V_L much like hapten molecules fit into the deep pocket described above. Thus there is a consistent involvement of the deep groove of the antibody-combining site. It can probably be expected that Ag–Ab interactions will fill the spectrum of binding modes, from the deep binding of hapten molecules, to the face-to-face interactions of the protein antigen interactions.

The exact physical interactions that cause a particular antibody to bind its ligand can only be inferred without a crystal structure. Several chemical techniques can be employed to estimate the most important elements of the binding interaction, however. Via this type of analysis, the physical interactions between antibody molecules and their ligands have been predicted to involve hydrogen bonds, salt bridges, hydrophobic interactions, and so on. In addition, it should not be presumed that the antibody or its ligand is a static structure. It has been proposed that either molecule may be induced to change its conformation in order to form an antibody–antigen complex (72).

Significance of Class Switch

As was indicated in an earlier section, the genetic organization of the variable and constant regions is such that the variable region can be linked to any of the constant regions (reviewed in ref. 73). As is presented in Chapter 14, the progression of expression from one class to another is regulated according to the differentiation state of the cell. The functional significance of this class switch is that a V region, specific for a particular antigen, can be expressed in the context of any of the effector functions associated with the various heavy chain constant regions. The selection of the ultimate heavy chain class that is expressed with a given variable region does not seem to be random, however. For example, in the mouse there is a significant correlation between anticarbohydrate specificities and the γ_3 subclass (74), and between antiprotein specificities and the γ_1 subclass. There is also a correlation between antiviral activity and the γ_{2a} subclass (75). The functional significance of these correlations is not clear. The mechanism by which this antigen-correlated class switch comes about is thought to involve the interaction with specific subsets of T cells and/or factors secreted by these cells.

IMMUNOGLOBULIN AS ANTIGEN

Before the widespread application of protein sequencing and the development of the techniques of molecular biology, the analysis of immunoglobulin structure was approached in a very different way. The most widely used tool to dissect immunoglobulin structure was the immune system itself. As a large glycoprotein, the immunoglobulin [either as total serum immunoglobulin or a homogeneous preparation (i.e., myeloma protein)] from an individual elicits a vigorous immune response when injected into another species or different members of the same species. Using this approach, several antigenic determinants of immunoglobulin structure were determined (review in refs. 11 and 12). The correlation of these determinants with structural features known today is remarkable.

When a rabbit is injected with a human myeloma protein, a heterologous antiserum is produced which specifically recognizes the immunizing myeloma protein. If a panel of other myeloma proteins is screened with this antiserum, some will react, and others will not. By creating a panel of antisera that recognize each of the myeloma proteins, it became clear that there were five basic classes of myeloma proteins. Two of these basic reactivities were split further, one into four distinct subclasses, and the other into two. These nine kinds of immunoglobulin are

found in the sera of all normal individuals, thus the term *isotype* (iso = equal) is used to describe them. Today we know that the five isotypes represent the five heavy chain classes; the reactivities that can be split further are the γ and α subclasses. The heterologous antisera used to define the isotypes react with the epitopes unique to the human isotypes. The antisera do not cross-react with all the isotypes because the determinants that are common between the isotypes are also common to rabbit immunoglobulin. By absorbing the anti-isotypic reagents with appropriate sera, reactivities that recognize light chain components can also be uncovered. Thus reagents can be generated to distinguish between the light chain types and subtypes as well as the heavy chain classes and subclasses.

Further dissection of the human myeloma proteins and more extensive studies on rabbit immunoglobulin revealed determinants similar to isotype, but with an important difference. These determinants were found on the antibodies of some individuals, but not all members of a species. In breeding studies with rabbits, it was shown that these determinants were inherited in a single autosomal dominant fashion. Thus an allelic marker of immunoglobulins was defined and subsequently termed *allotype* (allo = different). The determinants recognized by anti-allotypic antisera are more restricted than those of anti-isotypic antisera. Several allotypic determinants have been resolved to the level of the polymorphic amino acid interchanges that define them. Table 4 is a compilation of the known human allotypes, and where it is known, the amino acid substitutions that define these determinants are given.

Whereas an allotype is a determinant found in several, but not every, member of a species, an *idiotype* is an antigenic marker of an immunoglobulin which is generally found only in an individual member of the species. Idiotypes (idio = individual) were originally described via extensive absorptions of antisera specific for individual human myeloma proteins. It was shown that each myeloma protein bore antigenic determinants which were unique to that myeloma. The epitopes that define an idiotype are located in the variable region. An idiotypic determinant defined by a monoclonal antibody is called an idiotope.

Extensive research on idiotypes has revealed several interesting features of the immune response. In an individual's response to a particular antigen, the group of antibodies that are expanded have a common feature, that is, the ability to recognize the eliciting antigen. An antiserum against this original antiserum will therefore primarily recognize the variable regions of these expanded, antigen-reactive clones. This anti-idiotypic antiserum can be thought of as the "fingerprint" of an individual's immune response to a given antigen. In an inbred species (such as the inbred strains of mice) the immune response of each individual is very similar. Comparison of the immune responses of several inbred strains to the same antigen has revealed that while most strains will respond with their own particular predominant idiotype, minor components of the response may be idiotypically identical

TABLE 4. *Compilation of the human allotypes*[a]

	Type	Class	Allotype	Alphameric	Numeric	Position(s)	Amino Acid(s)
Light Chains	Kappa		Km 1			153, 191	val, leu
			Km 2			153, 191	ala, leu
			Km 3			153, 191	ala, val
Heavy Chains		IgG1	G1m	a	1	356, 358	asp, leu
				f	3	214, 219	arg, ser
				x	2	431	gly
		IgG2	G2m	n	13		
		IgG3	G3m	b0	11		
				b1	5	296, 436	phe, phe
				b3	13		
				b4	14		
				b5	10	419, 422	gln, ile
				c3	6		
				c5	24		
				g	21	291, 296	leu, tyr
				g5	28	419, 422	glu, ile
				s	15		
				t	16		
				u	26		
				v	27	422	ile
		IgA2	A2m	1			
				2		411, 428	tyr, glu
						458, 467	ile, ala
		IgE	Em	1			

[a] Where it is known, the position and identity of the amino acids involved in the allotypic determinants are indicated. From Weir (76) and Fudenberg et al. (77), with permission.

to the response of other strains. Thus idiotype is not necessarily unique to the individual. These shared idiotypes are known as cross-reactive idiotypes, and they generally represent the use of one or more common variable region gene segments. The mechanism(s) that regulates the idiotypic nature (i.e., the selection of particular V regions) of an immune response is an active area of research in several laboratories.

Anti-idiotypic antibodies are not merely the result of laboratory manipulations. Anti-idiotypic antibodies can be demonstrated in the sera of normal individuals and may have some regulatory role in the nature of the immune response. Evidence to support a functional role for idiotypy *in vivo* has been demonstrated by several laboratories. The idea that an anti-idiotypic antibody may regulate the expression of the corresponding idiotype, and hence the nature of the immune response, is the central concept of the network theory of immune regulation. Details of the network theory and the experiments that support it are presented in Chapter 23.

CONCLUSION

Simply from the length of this chapter, it should be clear that a great deal of information on the structure and function of immunoglobulin molecules has been determined. But for all this accumulated knowledge, there are a great deal of as yet unanswered fundamental questions. How does the binding of antigen signal the F_c region that complement can now be activated? How does the F_c region interact with complement to activate the classical complement cascade? What are the precise differences between the heavy chain classes that lead to the various biological properties which they possess? The list goes on and on.

With the continued application of the techniques of molecular biology, several of these issues will probably be resolved in the next few years, while others may never be known. In either case, the diversity of functions associated with antibody molecules, all based on the same simple three-dimensional structure, is a marvelous example of the power of molecular evolution.

NOTE ON VIEWING STEREO PAIRS

In order to get the full three-dimensional effect from the stereo pairs in this chapter you need to do one of two things:

1. If at all possible, obtain a pair of stereo viewing glasses. These are specially designed glasses for viewing stereo pairs; their use is relatively self-explanatory.
2. Learn to cross your eyes. Stereo pairs can be of two type: "cross-eyed stereo" or "wall-eyed stereo." These images are of the wall-eyed variety. When viewing a wall-eyed stereo pair, the idea is to allow your eyes to relax (out of focus even) and drift apart (i.e., toward the walls). As you do this, a second pair of images will appear between the pair of images on the page. By allowing your eyes to relax even more, this second pair of images will merge in the middle. Now, while keeping the images together, try to focus on this new middle image. You will find that this middle image is in three dimensions. With practice this will become easier.

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